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TABLE OF CONTENTS.

Page

115. The Medicinal Treatment of Parasitic Diseases—An Undeveloped Field of Veterinary Medicine. By Maurice C. Hall. (*The Journal of the American Veterinary Medical Association,* Vol. L, N. S. Vol. 3, No. 5, Jan., 1917, pp. 608-611) 1

116. Biological Standardization. By H. C. Hamilton. (*American Journal of Pharmacy,* Vol. 89, No. 2, Feb., 1917, pp. 61-70) 5

117. Bacterial Findings and Their Relationship to Pyorrhea Alveolaris and Interstitial Gingivitis. By A. W. Lescohier. (*The Journal of the American Medical Association,* Vol. 68, Feb. 10, 1917, pp. 414-416) . . 15

118. Comparative Study of Bacillus Anthracis-symptomatici and Allied Organisms with Respect to Gas-production. By L. C. Todd. (*The Journal of Infectious Diseases,* Vol. 20, No. 2, Feb., 1917, pp. 151-169) . . 21

119. Apparatus for Use in Examining Feces for Evidences of Parasitism. By Maurice C. Hall. (*The Journal of Laboratory and Clinical Medicine,* Vol. 2, No. 5, Feb., 1917, pp. 347-353) 41

120. The Longevity of Adult Ascarids Outside the Body of the Host. By Maurice C. Hall. (*The Journal of the American Medical Association,* Vol. 68, No. 10, 1917, pp. 772, 773) 51

121. Facts and Fallacies in Disinfection. By H. C. Hamilton. (*American Journal of Public Health,* Vol. 7, No. 3, March, 1917, pp. 282-295) 55

122. Canine Distemper: Review of the Work of the Various Investigators in the Attempt to Establish the Primary Etiological Factor. By N. S. Ferry. (*Proceedings of the Wisconsin Veterinary Medical Association,* 1917, pp. 80-88) 77

123. The Frequency and Significance of Dysfunction of the Internal Secretory System in the Feebleminded. By C. P. McCord. (*New York Medical Journal,* Vol. 105, No. 13, March 31, 1917, pp. 583-588) 85

Page

124. Digitalis Thapsi, Lin. By O. A. Farwell and H. C. Hamilton. (*American Journal of Pharmacy,* Vol. 89, No. 4, April, 1917, pp. 148-154) 101

125. The Pineal Gland—Influence upon Growth Differentiation and Prenatal Development. By C. P. McCord. (*Transactions of the American Gynecological Society,* 1917, pp. 41-63) 109

126. Evidences Associating Pineal Gland Function with Alterations in Pigmentation. By C. P. McCord. (*The Journal of Experimental Zoology,* Vol. 23, No. 1, May, 1917, pp. 207-224) 133

127. Desiccated Anthrax Antigen for Immunization Purposes. By N. S. Ferry. (*Journal of the American Veterinary Medical Association,* Vol. LI, N. S. Vol. 4, No. 2, May, 1917, pp. 200-203) 151

128. The Preparation and Standardization of Ovarian and Placental Extracts. By W. H. Morley. (*Transactions of the American Gynecological Society,* 1917, pp. 228-239) 155

129. Parasites of the Dog in Michigan. By Maurice C. Hall. (*Journal of the American Veterinary Medical Association,* Vol. LI, N. S. Vol. 4, No. 3, June, 1917, pp. 383-396) 165

130. Preparation of Protein Extracts for Diagnostic Cutaneous Tests. By N. S. Ferry. (*Journal of Laboratory and Clinical Medicine,* Vol. 2, No. 9, June, 1917, pp. 655-657) 179

131. Notes in Regard to Horse Lice, Trichodectes and Haematopinus. By Maurice C. Hall. (*Journal of the American Veterinary Medical Association,* Vol. LI, N. S. Vol. 4, No. 4, July, 1917, pp. 494-504) ... 183

132. The Susceptibility of the Prairie Dog to Rabies. By G. I. Walters. (*Journal of the American Veterinary Medical Association,* Vol. LI, N. S. Vol. 4, No. 5, Aug., 1917, pp. 702-704) 195

133. Manure Disposal as a Factor in the Control of Parasitic Diseases of Live Stock. By Maurice C. Hall. (*Journal of the American Veterinary Medical Association,* Vol. LI, N. S. Vol. 4, No. 5, Aug., 1917, pp. 675-678) 199

Page

134. The Rationale of Pollen Extract Therapeusis of Hay Fever, with a Report of 127 Cases. By N. S. Ferry. (*Interstate Medical Journal,* Vol. 24, Sept., 1917, pp. 884-893) 203

135. The Influence of the Method of Administration Upon the Degree of Toxicity of Strophanthus Preparations. By L. W. Rowe. (*The Therapeutic Gazette,* Vol. 33, 3d S., No. 8, Aug., 1917, pp. 536-540) 215

136. Coccobacillus (Foetidus) Ozaenae of Perez. By Herbert C. Ward. (*The Journal of Infectious Diseases,* Vol. 21, Oct., 1917, No. 4, pp. 338-340) 225

137. Notes in Regard to Bots, Gastrophilus SPP. By Maurice C. Hall. (*Journal of the American Veterinary Medical Association,* Vol. LII, N. S. Vol. 5, No. 2, Nov., 1917, pp. 177-184) 231

138. An Investigation of the Chemical Composition and Biologic Availability of Peptone. By L. Davis. (*Journal of Laboratory and Clinical Medicine,* Vol. 3, Nov., 1917, No. 2, pp. 75-86) 239

139. Anthelmintic Treatment for Nematode Infestations in Dogs. By Maurice C. Hall. (*Journal of the American Veterinary Medical Association,* Vol. LII, N. S. Vol. 5, No. 3, Dec., 1917, pp. 342-345) .. 255

140. The Hypodermic Use of Iron. By L. W. Rowe. (*The Therapeutic Gazette,* Vol. 33, 3d S., No. 12, Dec., 1917, pp. 847-849) 259

141. The Value of Yeast Vitamine Fraction as a Supplement to a Rice Diet. By A. D. Emmett and L. McKim. (*The Journal of Biological Chemistry,* Vol. 32, No. 3, Dec., 1917, pp. 409-419) 265

142. New Species and Varieties from Michigan. By O. A. Farwell. (*19th Mich. Acad. Sci. Rept.,* 1917, pp. 247-250) 277

143. Rare or Interesting Plants in Michigan. By O. A. Farwell. (*19th Mich. Acad. Sci. Rept.,* 1917, pp. 251-262) 281

144. Hog Cholera Transmission Experiments. By H. Preston Hoskins. (*Proceedings of the Missouri Valley Veterinary Association,* 1917, pp. 123-135) 293

113348

Studies from the Research Laboratory
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THE MEDICINAL TREATMENT OF PARASITIC DISEASES—AN UNDEVELOPED FIELD OF VETERINARY MEDICINE.*

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With the exception of those parasitic diseases which are of interest from the standpoint of quarantine regulations (Texas fever and scabies) or of meat inspection (hydatid, "beef measles," "hog measles," etc.), the diseases of stock due to parasites receive entirely too little attention from the veterinarian.

One reason for this is that such animals as sheep and swine, which animals most commonly suffer from parasitic infestation, are species with which the veterinarian has little to do, aside from dipping sheep for scabies and injecting hogs with serum against hog cholera. That veterinarians treat sheep and hogs so little is due to a combination of circumstances. From the farmer's standpoint, the value of the individual sheep or swine commonly does not warrant the expense of paying a veterinarian. From the veterinarian's standpoint, the compensation to be expected from treating the individual sheep or swine does not warrant an interest in this field. For years veterinary practice in the United States concerned itself primarily with horses and secondarily with dogs. Today cattle practice is coming into its own, but aside from hog cholera work, sheep practice and swine practice are little known quantities in the United States.

The field of sheep and swine practice is one that deserves attention. The diseases that attack these animals are commonly epizootic or enzootic, and the aggregate value of the stock involved is usually considerable, even though the value of the individual animal be comparatively small. At the present time the value of even the individual sheep or swine is no small matter.

Sheep, swine and dogs are commonly infested with worms.

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Sheep, especially, are affected but little by bacterial diseases and suffer principally from parasitic infestation. Quite often the animals suffer from massive infestations with the usual damage due to irritation of the digestive mucosa from the movement and pressure of the worms, from occlusion of the lumen of the digestive tract, from enterospasm, from abstraction of nourishment, from absorption of toxic products, and from hemorrhage and traumatic injury occasioned by some species of worms. The damage is most pronounced in young animals. In these animals the digestive tract is more sensitive, the mucosa more tender, the reflexes perhaps more acute. Infestations that give rise to little or no apparent injury in old animals often appear to occasion considerable injury to young animals and in some cases are the cause of death. One of the most important features in the case is the fact that young animals that are heavily parasitized commonly suffer a serious setback and frequently are permanently stunted in growth. They become unthrifty adults—"runts."

Clinically, parasitic infestation does not present a sharply defined, well characterized picture, but in a general way one may suspect parasitism when dealing with an afebrile disease, with unthriftiness grading into emaciation and commonly accompanied by anemia and edema. Gastric and intestinal parasitism is often accompanied by more or less diarrhea or constipation. These features should be considered in relation to other facts: that parasitic worms are most prevalent in warm climates and moist localities or in wet years; that they are, therefore, more apt to be of importance in the South and along the coast than in the Rocky Mountain States or in such dry climates as parts of Arizona; and that continued use of closely cropped pastures will favor massive infestation. As a rule there is opportunity for post-mortem examination to confirm the suspicion of parasitism as the cause of trouble, for the veterinarian is not apt to be called in until the death of at least one animal makes it seem desirable to have advice. Of course, it is possible to confirm a suspicion of parasitism by microscopical examination, but at the present time there are comparatively few practicing veterinarians sufficiently experienced to make a satisfactory diagnosis in this way, especially when it involves the question of passing on whether the fecal findings indicate a common and light or moderate infestation or a massive and dangerous infestation, a rather difficult point to settle. To be sure, the presence of parasites would warrant treatment from many standpoints, but it would not necessarily settle the question as to whether they were responsible for the condition the veterinarian was called in to treat.

Most of the work on parasitic diseases of live stock in this country has been done in the federal bureaus. From the very nature of things they have, therefore, laid most of their emphasis on prophylaxis, something that could not well be over-emphasized. They have, however, noted the necessity and desirability of medicinal treatment, and it is well known that there are times when such prophylactic measures as pasture rotation are, for one reason or another, not feasible. An illustration of this fact that came to the writer's attention lately was in the case of two flocks of goats used as brush cleaners on Virginia farms. It was necessary that the goats feed down the brush to the point where it would die, a feature which made it undesirable to rotate pastures and give the brush which had only been lightly pastured a chance to recuperate. In cases like this the repeated use of proper medicinal treatment might reasonably be expected to gain all that pasture rotation would gain in the way of keeping infestation down to a negligible minimum.

There is an excellent opportunity at the present time for the veterinarian to educate the owners of sheep and swine in regard to the desirability of employing veterinarians to treat their stock for parasitic diseases. Live stock values are especially high at the present time, and there is less than the usual amount of philosophical resignation in contemplating dead stock. Live stock papers and farm journals are emphasizing more and more the importance of worm parasites and related pests and the need of combating them. If the veterinarian does not take occasion to interest himself in this field at the present time, he will sooner or later find himself lamenting the prevalence among farmers and stockmen of the practice of treating their own flocks and herds for parasites. There is a growing interest in this matter which marks the veterinarian's opportunity to take advantage of the situation or give rise to a regrettable situation by neglecting it. If the farmer could treat his stock as well as the veterinarian could, it would only be the veterinarian's loss if the farmer became confirmed in this practice. But this would not be the case. The veterinarian is in a position to know the latest, safest and best treatments, whereas the farmer, who cannot be so well informed, is apt to waste time and money and lose stock by the inexpert administration of ineffective or dangerous treatments.

There are doubtless many veterinarians who feel that the task of dosing a flock of sheep or a herd of swine could not possibly be accomplished in sufficient time to make it remunerative. More experience will dispel this idea. With three or four assistants, such as can usually be found on a sheep ranch, flocks of five hundred to perhaps a thousand sheep could be drenched in a day, by the use of suitable equipment, at a cost which would make it profitable to the veterinarian and the sheep owner. This means that the sheep must be drenched at a rate of more than one animal a minute, and in the writer's experience it is not especially difficult to work at that rate. With proper facilities for handling and restraint, swine can also be handled at a rather rapid rate.

The stomach worm has ruined the sheep industry in some sections. The practicing veterinarian owes it to himself and to the sheep business to cultivate an interest in the stomach worm problem instead of neglecting it. And what is true of the stomach worm is true, in a general way, of other parasitic diseases which cannot be discussed within the limits of this paper. Such prophylactic measures as pasture rotation must be carried out by the farmer and stockman, preferably under the advice of a trained veterinarian, but no adequate scheme of eradication or control can neglect medicinal treatment, and this is and should remain the veterinarian's field. But the saving of stock is the object which gives point to the veterinarian's work, and if he refuses to take an interest in sheep and swine and to educate his community in regard to the possibilities of treatment and the need for treatment of parasitic diseases by the skilled veterinarian, then the stock journals will develop in the farmer and stockman the idea that they must treat their own stock for these troubles.

Studies from the Research Laboratory
Parke, Davis & Co.
Reprint No. 116, 1917

BIOLOGICAL STANDARDIZATION.

By Herbert C. Hamilton.

(From the Research Laboratory of Parke, Davis & Co., Detroit, Michigan.)

Under this heading Rusby (1) reviews the U. S. P. methods for standardizing the drug and glandular extracts which admit of no exact chemical assay. He asserts that from the viewpoint of the medical profession any favorable opinion regarding these methods is almost entirely because of "confidence in those who recommended them." But that even "this confidence was considerably weakened because of radical differences which existed among those authorities." The parallelism between therapeutic value and the measured physiological effect was also questioned. "On the other hand the convention had the greatest confidence in the specialists who were in charge of these investigations."

The review while entirely impartial is lacking in one important feature: it fails to give the viewpoint of the manufacturer just as the revision committee failed to give it due consideration. The importance of this feature is in the fact that the manufacturers were in many instances the originators of the tests in use, and had of necessity developed them from scientifically interesting facts to a practical working basis. These manufacturers through years of experience were in a position to eliminate the non-essentials and to arrive at some common ground by compromise. A method which under certain conditions can be made fairly satisfactory may fail entirely when attempts are made to apply it under practical working conditions.

As may be inferred from the foregoing introduction it is the purpose of the writer to point out certain objectionable features in the biological assays which are either suggested or required in the 9th Revision of the U. S. Pharmacopœia (2).

We will assume, as the Revision Committee evidently did, that a biological method can be found which is a measure of therapeutic value.

It is very natural that opinions should differ among pharmacologists as to which reaction more nearly represents the activity of a drug, when the drug has a number of apparently different therapeutic uses.

It is no more remarkable that differences of opinion exist as to which is more accurate, if several assay methods are available. One can expect therefore that the methods adopted would be subjected to criticism, for in this way only can errors be eliminated.

THE DIGITALIS SERIES OF HEART TONICS.

The method adopted for the assay of the digitalis series of heart tonics is that known as the one-hour frog heart or the minimum systolic dose (M. S. D.) method.

In a review of the various methods in use Edmunds and Hale (3) eliminated most of them as illogical or inaccurate. These authors found little reason for choice between the one mentioned above and another known as the minimum lethal dose (M. L. D.) method.[1] Their preference inclined toward the former on the basis of cost and time.

This method leaves much to be desired in regard to accuracy and dependability, since if one economizes either in time or in material it is usually at the expense of accuracy.

This method is subject to the same variable factors as the M. L. D. method, namely, temperature, season, different species of frogs and their individual susceptibility, with the additional disadvantage that absorption becomes a more serious factor on account of the shorter time of action. The apparent advantages—fewer frogs and shorter time—are under some conditions real disadvantages because in case of delayed absorption a frog's heart will be found beating in 1 hour, even when dosed with the M. S. D., while the M. L. D. would not be appreciably affected. Another important point in connection with the end point in the M. S. D. method is the effect of pithing the frog and laying bare its heart. Is it reasonable to suppose that this has no influence on the condition of the heart? Whatever this influence is it can scarcely be inferred that this is an invariable and uniform effect. The method therefore introduces one more factor, the effect of which tends towards variability rather than in the direction of increasing accuracy.

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[1] The M. L. D. or minimum lethal dose method is described in detail by Houghton and Hamilton (4). The M. L. D. is the smallest dose per gram weight of frog which kills a majority of the test animals, the correctness of the end point being verified by observation of the laid-bare heart, which is in systole, if death is due to the uncomplicated action of one of the digitalis series.

After an extended series of tests Hamilton & Rowe (5) found that the M. S. D. of digitalis is less than the M. L. D., showing that an early paralysis of the heart occurs which may be the cause of the observed indefinite end point. This paralysis seems to have no uniform relationship to the M. L. D. since in some cases the M. L. D. and M. S. D. were identical, while an average of 14 experiments showed the latter to exceed the former by 22 per cent. in the case of fluid extracts, and 36 per cent. in the case of tinctures, the latter being an average of 12 tests. In one assay of a tincture by both methods the M. L. D. exceeded the M. S. D. by 60 per cent.

The conclusion of Edmunds and Hale that choice between the two methods is largely one of convenience seems unwarranted. The most uniform end point, and the most logical, is that where the sample of a digitalis preparation has an opportunity to complete its cycle of effects and to be measured by the size of the minimum dose which causes death of the frog with heart in systole. Any other stage in this cycle is more variable and therefore less accurate as an end point for the reaction.

The standard, ouabain, adopted by the committee for comparison in measuring the activity of the digitalis series of heart tonics is also open to criticism because it is obtained from a non-official strophanthus seed and because different lots are not uniform in composition.

While it is true that the standard, in physiological assaying, is merely to measure the resistance of the frogs, this resistance is of such a complex character that it should be measured by a standard, not in any respect open to criticism. The standard, if not identical with the sample, should be one whose composition and identity have been established.

The description of ouabain indicates that it is derived from a non-official strophanthus seed, that its composition is indefinite in that it crystallizes with varying quantities of water, and that it does not yield a crystalline strophanthidin and cannot therefore be assayed chemically to establish uniformity. That it is not uniform is shown by Rowe (6), a conclusion which may be deduced from the fact that the M. S. D. of ouabain accepted by the U. S. P. Committee is .0000005, while the average of the three samples tested by Rowe is .00000086 or 76 per cent. more—a difference not due to temperature, because in all cases the tests were carried out at 20° C. Further it is an expensive substance and obtainable only by importation. These and perhaps other objections would certainly have been brought out by the ones who must use the test if such an opportunity had been granted.

Strophanthin from Kombe seed can be made of uniform composition and activity according to Brauns and Closson (7) and is therefore preferable to ouabain for every reason, but its use seems especially logical because of being derived from the official strophanthus seed.

Cannabis.

The biologic assay of cannabis indica is another that is open to criticism, not only in the wording of the text, but in the size of the test dose and in certain of its details.

As to the words used, the statement is made that cannabis "produces incoördination when administered to dogs in a dose of not more than 0.03 mil of fluid extract, ..." How much smaller dose will show a similar but less marked effect? What is the effect of a larger dose? A logical wording would be that Standard F. E. Cannabis produces incoördination in a dose not less than, etc.

The dose chosen is too small for a satisfactory test. The average dog will scarcely react to a dose of this size, and if the drug is somewhat less active than standard, the animal will not react visibly. Under the rather drastic conditions imposed, some dogs will show the effect of cannabis to a fairly satisfactory degree, but a large majority will show the effect very slightly if at all—a statement corroborated by Pearson (8).

A much more satisfactory test dose of a standard cannabis preparation is one which when administered under proper conditions will show a well-marked action. Then if the preparation being tested is somewhat less active than standard or the dog slightly less susceptible than normal, an evident although less marked effect is produced. In this way a measurable difference can often be established, a difference which can be verified by a second test. A dose of 0.1 mil of the standard fluid extract does not produce an effect too great to obscure the reaction of a sample which is slightly more active than standard. This dose is therefore recommended as the standard test dose. In a former communication the author (9) suggested 0.01 Gm. as the dose for the extract and a minimum dose of 0.09 mil (Cc.) of the fluid extract.

Testing the standard on each occasion is an unnecessary and expensive procedure, since a dog proved to be susceptible to the standard to the proper degree remains fairly constant for a long period, if in good physical condition. This is admitted in the official description of the test animals (2).

Testing a sample on two dogs which have been chosen because of being susceptible to the standard test dose of cannabis is sufficient unless one or both exhibit a reaction very different from that to be expected. In such a case the susceptibility of the dogs should be reëstablished.

Starving a dog for twenty-four hours is a longer period than necessary. Twelve hours is generally sufficient to empty the stomach, and is less trying on the test animals.

Regarding the standard, some more exact method should be devised for obtaining uniformity among manufacturers. As prescribed by the U. S. P. the activity of a sample depends on the susceptibility of the dog, and it may be any kind of a dog.

If a test is attempted for the first time and no reaction follows, how can it be determined whether the sample or the animal is at fault?

Since the test is compulsory it should be the duty of some central authority to supply a sample which meets requirement to every manufacturer of cannabis products, and so insure the uniformity which is planned to attain.

It is an unnecessary requirement to compare each cannabis preparation with the standard in the same form. If the standard is in a stable form it can be considered equally active if .004 Gm. is in extract, fluid extract or tincture form, while in fact the statement "A standard fluid extract will produce incoördination when administered to dogs in the dose of 0.03 mil, the extract in the dose of 0.004 Gm., the tincture in the dose of 0.3 mil for each kilogram of body weight of dogs," gives one no option in dosing, and practically makes a test of the standard unnecessary. The effective dose of each is prescribed without any opportunity of varying it to adjust for a lower susceptibility of the test animal. If the method provided for this contingency, it would be logical to compare sample with standard in every case and to eliminate every possible cause for unequal effects.

Further criticisms of this test of cannabis preparations are that the drug, while intoxicating in a sense, is not known to be fatal in a relatively immense dose and consequently is not a dangerous drug; and that it has no very extended application in human therapy. It therefore scarcely deserves so much attention, especially when the extra refinements of testing suggested add so little to uniformity in the activity of its extracts and so much to the cost of standardizing.

Suprarenal Gland.

The biologic assay of products of the suprarenal gland is open to criticism in only two particulars, namely, in the method of measuring and administering the doses and in attempting to check the results as described.

Using both femoral veins for injecting sample and standard alternately is probably intended to obviate the possible mixing of the two solutions if both are injected into the same vein. But it introduces a very much greater source of error. The amount injected can much more easily be measured by use of a pipette than in a syringe, and the dose after being injected can be easily and completely washed into the blood stream by a follow-up injection of 2 mil (Cc.) physiological salt solution. When this procedure is followed, no mixing of two injections is possible.

By the U. S. P. method it is difficult to measure accurately small differences in the dose, and impossible to insure that the entire dose enters the blood stream. The small part remaining in the dead space of the vein seems to be destroyed, because doses administered by the U. S. P. procedure are invariably less effective than when immediately and completely carried into the blood stream by the method suggested above.

The requirement of checking an assay by making the injections of sample and of standard into the opposite sides from those first used is no check except in so far as it checks conditions on the two sides of the dog. This feature can better be eliminated by using only one side. Further, by the official method, if it is impossible to complete the test and the check on a dog, no option is left but to repeat both test and check on another dog. It is occasionally necessary to check an assay on a second dog when conditions during the first test were unfavorable for accuracy, but no advantage results from a retest on the same dog.

Pituitary Gland.

The assay of liquor hypophysis, while not included in the biological assays on page 601 *et seq.* is none the less biologic. It is also compulsory for a U. S. P. product. It is open to criticism because of the nature and dose of the standard and because of the unsatisfactory character of the test reaction chosen.

First as to the standard, it seems unwise as well as unnecessary to choose as the standard a substance which has only one of the typical physiological effects of hypophysis, and which alone has no therapeutic application equivalent to that of extracts of the pituitary gland.

In a previous communication (10) the author described the means of obtaining a satisfactory standard test solution, this being a fresh solution of the water-soluble part of the dried, defatted, powdered infundibulum.

Because of the possible variation in the content of active agent in different lots of glands, a stable, highly active, water-soluble powder prepared by Aldrich (11) has been largely substituted in the commercial standardization of pituitrin. The dried glandular substance first mentioned, however, can be easily prepared and is well adapted to the purpose of making the standard test solution.

This has the distinct advantage over histamine of being identical in action with extracts of the glands both on the isolated uterus and on other unstriated muscles, especially those controlling blood pressure.

Active extracts of the pituitary gland are remarkable for their action on smooth muscular tissues, contracting the uterus and intestinal muscles and the walls of the veins and arterioles. As results of this action, the blood pressure is raised, peristalsis is increased and the tone of an inert uterus is aroused.

It is logical to assume that all the effects are due to a constituent which acts on unstriated muscle and to choose one of these as a test reaction—one which is accurate and measurable. If, however, the action on the pregnant uterus is due to a constituent other than that which affects the blood pressure, then it is essential that the oxytocic test be applied to those preparations intended for obstetrical work. But for the assay of such as are used to control the blood pressure in cases of shock it would be necessary to apply the blood pressure test.

While some investigators, notably Fühner (12), claim to have produced evidence that two or more constituents are present in an active extract, others have found the two principal effects to go hand in hand, pointing either to the presence of only one active constituent or to a constant ratio in the content of the two constituents. Roth (13), whose work was probably the basis for the choice both of the method and the standard test dose, submitted little conclusive evidence for his choice of the oxytocic method. He made his selection because "the simplicity of the isolated uterus method, together with the fact that the uterus of the virgin guinea-pig shows little variation in its reaction to pituitary extracts even after hours, places it above other methods in point of desirability as a method." His objection to the blood pressure method is "that one encounters a degree of tolerance when a second or succeeding dose is given."

On the latter point he submits as evidence of toleration a series of 14 consecutive injections of pituitrin, only 9 of which, however, illustrate his point. Each of the first five was followed by an almost identical rise in pressure, while the others were made in a manner contrary to his own suggestion, namely, "the period between injections to be at least 30 minutes long." The nine injections were made with an average interval of 5 minutes and into a dog which was apparently incompletely anesthetized. He submits no evidence whatever on the point that "little variation is shown in the reaction of the isolated uterus to pituitary extracts even after hours."

While the method is apparently simple the test strips of uterus muscle which will respond differently to different sized doses with any degree of accuracy are so few that a dependable test can rarely be made. On the other hand a blood-pressure test can be made on dogs with few exceptions and the "tolerance" referred to is scarcely to be observed when testing a good, carefully prepared extract. It can be eliminated entirely as a factor by the alternate injection of sample and standard. These facts are brought out and illustrated by Hamilton & Rowe (14), who suggest that clinicians working in conjunction with pharmacologists aid in determining which reaction more nearly coincides with therapeutic value. No positive evidence has yet been brought forward to prove that the blood pressure test carefully carried out is not a direct measure not only of pressor, but also of oxytocic activity together with its action on the kidneys, intestines and the mammary glands.

When a therapeutic agent such as the extract of the pituitary gland has been in practical use for nearly ten years and through clinical as well as laboratory experiments has been standardized to a certain degree of activity—a strength demanded by obstetricians and surgeons as essential for obtaining the desired results—any readjustment of activity should be made with extreme caution.

The revision committee, however, ignored this phase of the question and have adopted a standard activity for Liquor Hypophysis U. S. P. 9th considerably below that of the standard preparations on the market.

To make an official extract from this gland material conforming in every respect to U. S. P. requirements lowers the standard from that at present generally used by the medical profession to one with only 40 per cent. as high activity. Is there not reason enough for Dr. Rusby's statement that "it may safely be said that its (biological standardization) efficiency and authority were seriously doubted by a large majority of the medical profession?"

It is in many cases difficult enough to answer the question "Is the sample of drug that has been found to possess the greatest power to kill a cat the one that will prove most efficient in curing a man?" The advocates of biological standardization are now in the position where they must either ignore the official "biological assays" or be forced to justify methods which are in part at least illogical and inaccurate and standards which are not in line with medical requirements.

Considering the present status of biological standardization this is an unfortunate situation.

Summary of Objections to the Official Biological Assay Methods.

The Heart Tonics.

1st. The inaccurate method because the end point is obscured by (*a*) variable rate of absorption, (*b*) shock in exposing the heart.

2d. The standard, because it is not from the official drug and is not uniform in composition or activity.

Cannabis Sativa.

1st. The inaccurate wording in the text.

2d. The smallness of the test dose.

3d. The absence of a uniform standard.

4th. The non-essential features which add to the complications of the method with no commensurate gain in the activity or uniformity of the product.

Suprarenal Gland.

The complications introduced into the test:

(*a*) The inaccurate manner of measuring the test dose.

(*b*) The incomplete administration of the test dose.

(*c*) The method of making a check assay.

Pituitary Gland.

1st. The inaccurate and unsatisfactory character of the method.

2d. The standard, which is not adapted to measuring blood pressure activity, is not a practicable oxytocic agent in therapeutics and is not derived from the pituitary gland.

3d. The activity of the standard product.

BIBLIOGRAPHY.

1. Rusby. Druggists Circular, Sept., 1916.
2. U. S. Pharmacopœia, 9th Revision, p. 604 et seq.
3. Edmunds & Hale. Hygienic Laboratory, Bulletin No. 48.
4. Houghton & Hamilton. *Am. Jour. of Pharmacy,* Oct., 1909.
5. Hamilton & Rowe. *J. A. Ph. A.,* Jan., 1915.
6. Rowe. *J. A. Ph. A.,* Nov., 1916.
7. Brauns & Closson. *J. A. Ph. A.,* 1913.
8. Pearson. *J. A. Ph. A.,* Nov., 1916.
9. Hamilton. *J. A. Ph. A.,* March, 1912.
10. Hamilton. *J. A. Ph. A.,* Oct., 1912.
11. Aldrich, T. B. Patent Application.
12. Fuhner. *Zeitschr. f. d. ges. Exp. Med.,* 1913, I, 397.
13. Roth. Hygienic Laboratory, Bulletin No. 100.
14. Hamilton & Rowe. *J. Lab. and Clin. Med.,* Nov., 1916.

101483

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BACTERIAL FINDINGS AND THEIR RELATIONSHIP TO PYORRHEA ALVEOLARIS AND INTERSTITIAL GINGIVITIS *

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Few subjects in medical history have given rise to more frequent controversies than the inflammatory diseases of the gums. The classification, nomenclature, pathology, even the anatomy of the parts involved have afforded material for protracted argument. Probably no phase of the subject has been more keenly discussed than the etiology, and during recent years much of this interest has centered on the part played by bacterial invaders.

The probable relationship of pathogenic bacteria to the development of pyorrhea has been recognized for several decades. Mallasez and Galippe[1] (1884), Black[2] (1887), Miller[3] (1889) and Kirk[9] (1898) were some of the early investigators of its bacteriology. A variety of organisms were described, none of which could be specifically associated as the causative factor. Probably the first to ascribe etiologic significance to any particular micro-organism was Arkövy[5] (1904), who attributed acute cases of alveolar suppuration to streptococcic infection.

A detailed review of the voluminous literature on the bacteriology of pyorrhea and allied conditions appears neither necessary nor desirable. Many of the reports were based on studies the scope of which was limited both by the paucity of cases under observation and the facilities and training for their intelligent investigation. It seems advisable rather to try to correlate the facts established by those investigators who have definitely contributed to the bacteriology of the disease.

Perhaps the most notable of the early studies on the relation-

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* Read before the Section on Stomatology at the Sixty-Seventh Annual Session of the American Medical Association, Detroit, June, 1916.

1. Mallasez and Galippe: Compt. rend. Soc. de biol., 1884.
2. Black: Indep. Pract., New York, August, 1887.
3. Miller: Die Mikroorganismen Mundhöhle, Leipzig, 1889.
4. Kirk: Dental Cosmos, 1898.
5. Arkövy: Brit. Med. Jour., 1904, **2**, 1371.

ship of microörganisms to the pathology of pyorrheal diseases is that of Goadby.[6] His first report in 1907 describes a variety of organisms occurring in connection with pyorrhea, to none of which it was possible to attribute a definite etiologic relationship. This group of organisms included, in addition to the pyogenic cocci, various types of bacilli and spirilli. Curiously enough, in view of later developments and the fact that a study of his protocols shows that streptococci and staphylococci were found much more frequently, Goadby appeared to attach considerable importance to the bacillary forms. In 1908 Goadby reported the successful treatment of two acute cases of pyorrhea alveolaris with autogenous vaccines; in one of these the infection was mixed streptococcus and *Staphylococcus albus*, and in the other streptococcus was present almost in pure culture. In 1909-1910 a supplementary study of seventy cases was reported, in which the findings are given in Table 1.

During the period from 1907 to 1912 articles dealing with the bacteriology of pyorrhea were contributed by numerous workers, among whom may be mentioned Carmalt-Jones and Humphrey,[7] Sims,[8] Eyre and Payne,[9] Fletcher,[10] Beebe,[11] Best,[12] Williams,[13] Logan,[14] and Leary.[15] The results of their investigations were diverse and do not materially assist in clearing up the confusion existing relative to the bacteriology of the disease. The only deduction which they justify is that a number of microörganisms, potentially pathogenic, may under certain conditions be isolated from suppurative lesions of the gums.

The possibility of spirochetes being concerned pathologically in pyorrhea has received some attention. Various workers, including Noguchi, have described spirochetal types found in the oral cavity, and in 1912 Noguchi[16] isolated, in pure culture, from a case of pyorrhea a mucin-producing spirochete which he named

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[6] Goadby: Brit. Med. Jour., Sept. 9, 1905; Tr. Odontological Soc., March, 1906; Lancet, London, March 9, 1907; Brit. Med. Jour., Aug. 22, 1908; Lancet, London, Dec. 25, 1909; Practitioner, London, 1912, **88**, 107.

[7] Carmalt-Jones and Humphrey: Lancet, London, Dec. 28, 1907.

[8] Sims: Dental Practitioner, Toronto, October, 1908.

[9] Eyre and Payne: Proc. Roy. Soc. Med., Odontological Section, Nov. 22, 1909.

[10] Fletcher, M. H.: Unclean Mouth and Its Evil Results, The Journal A. M. A., July 13, 1912, p. 94.

[11] Beebe: Boston Med. and Surg. Jour., Oct. 28, 1909.

[12] Best: Dublin Jour. Med. Sc., **133**, 91.

[13] Williams: Am. Jour. Med. Sc., 1911, **132**, 666.

[14] Logan: Dental Rev., 1911, **25**, 649.

[15] Leary: Dental Cosmos, 1909, p. 55.

[16] Noguchi: Jour. Exper. Med., 1912, **16**, 194.

Treponema mucosum. This organism is not regarded by Noguchi as strictly parasitic, but he ascribed to it pyogenous action under conditions in which the tissues have been so injured as to enable it to survive. The strong fetid odor in some cases of pyorrhea he believes to be due in part at least to the presence of *T. mucosum.*

Recent bacterial studies which seem to be entitled to careful consideration are those reported by Medalia,[17] Brown,[18] and Hartzell.[19]

TABLE 1.—ORGANISM FINDINGS IN SEVENTY CASES OF PYORRHEA ALVEOLARIS.

Organisms	No. Cases Present
Streptococcus	19
Pneumococcus	5
B. necrosis-dentalis	14
M. catarrhalis	23
B. septus	15
Staphylococcus aureus	12
Saccharomyces	5
Micrococcus citreus-granulosis	6

Medalia's reports were based on a study of 115 cases, in which a careful bacteriologic investigation appears to have been carried out. The series included fourteen incipient, sixteen moderately advanced, and eighty-five advanced cases. The findings reported are as given in Table 2.

We would be led to infer from these results that the pneumococcus is the most important organism to be considered, as it was found either alone or in combination in 107 of the 115 cases (93 per cent), and the streptococcus only an occasional invader, as it was reported as being present in only fourteen cases (12 per cent.). The work of other investigators does not, however, substantiate this deduction. Brown in forty-eight cases observed *Streptococcus viridans* twenty-three times, hemolytic streptococci thirty times, and non-hemolytic streptococci twenty-eight times. Pneumococci were present in twenty-seven cases, staphylococci, diphtheroids and organisms of the *Bacterium influenzae* group being also frequently found.

Hartzell's work, perhaps the most convincing of any that has been reported, points strongly to the importance of the streptococcus in pyorrhea. His observations have indicated that approximately three-fourths of the bacterial content of pyorrhea pus pockets is made up of pyogenic cocci of the *Streptococcus viridans,* and staphylococcus types, and the remaining one-fourth of other organisms. Hartzell's animal inoculations have shown that following the intravenous injection of pus from pyorrhea pockets streptococci of the viridans type are usually recovered, occasionally staphylococci.

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[17] Medalia: Boston Med. and Surg. Jour., **167,** 868, 922; Dental Cosmos, January-February, 1913.

[18] Brown: New York Med. Jour., 1915, **98,** 1201.

[19] Hartzell: St. Paul Med. Jour., 1916, **18,** 77; Report First District Dental Society of the State of New York, Jour. Allied Dental Societies, March, 1916.

Personal observations relative to the occurrence of streptococci, staphylococci and pneumococci in pyorrhea would place streptococci first in frequency, staphylococcus next, pneumococci being observed in only a small percentage of cases.

The scope of this paper does not extend to a consideration of the relationship existing between pyorrhea and systemic diseases. The importance of oral infection in metastatic infections of the heart, blood vessels, joints, kidneys and other tissues is, however, generally recognized. Hartzell has emphasized the fact that of the organisms found in the mouth, there are none having the varied possibilities for disease production characteristic of the streptococcus group. Clinical observations as well as the results of animal inoculations have clearly shown that in the remote pathologic effects of oral infection, the streptococcus overshadows in importance all other organisms which may be present in pyorrhea pockets.

A discussion of the causative factors in pyorrhea necessarily involves a consideration also of its pathology. Considering interstitial gingivitis and pyorrhea alveolaris as phases of the same disease, we generally recognize that bacterial invasion is characteristic of the pus stage and is not necessarily present in cases of interstitial inflammation without pus. The bacterial studies which have been reported are based largely on investigation of cases of the purulent type. The occurrence of gingival inflammation, without infection, as the result of trauma, irritation and constitutional influences during the non-purulent inflammatory stage of the disease is scarcely open to question. On the other hand, the conclusion of some investigators that bacterial invasion in pyorrhea is invariably a secondary factor, infection being universally absent prior to the pustular stage of the disease, does not seem to be established. An infected zone around the root of the tooth, usually due to acute streptococci infection, may exist. It would be strange indeed if this were not so, since the poorly protected gingival crevice offers a splendid opportunity for bacterial attack, the microbic penetration being aided by the formation of calculi on the roots of the teeth or at the gingival margin and the number of the infecting organisms being augmented by the neglect of oral hygienic measures.

TABLE 2.—BACTERIAL FINDINGS BY MEDALIA IN 115 CASES OF PYORRHEA ALVEOLARIS.

	No. of Cases with Organisms	Per Cent.
Incipient Cases (14)		
Pneumococcus and staphylococcus	10	71
Pneumococcus	2	14
Staphylococus and streptococcus	1	7
Sterile	1	7
Moderately Advanced Cases (16)		
Pneumococcus and staphylococcus	8	50
Pneumococcus	6	37
Staphylococcus	1	6
Sterile	1	6
Far Advanced Cases (85)		
Pneumococcus and staphylococcus	49	57
Pneumococcus	18	21
Staphylococcus	1	1÷
Pneumococcus and streptococcus	3	3÷
Pneumococcus, streptococcus and staphylococcus	10	12
Pneumococcus and Micrococcus catarrhalis	1	1÷
Staphylococcus and Micrococcus catarrhalis	2	2÷
Sterile	1	1÷

The tendency to consider any etiologic factor in pyorrhea as an entity instead of in relationship to other influences is unfortunate. Irritation, trauma, metabolic disturbances and microbic invasion constitute a vicious circle in the development of alveolar diseases. The bacterial element is probably the most important element in the destructive tissue changes and certainly so in the serious sequelæ. While it is impossible definitely to classify interstitial gingivitis or even pyorrhea as primarily a microbic disease, the pathology is intimately associated with the results of bacterial invasion and the adequate consideration of its treatment must necessarily recognize the importance of the infectious element.

101481

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COMPARATIVE STUDY OF BACILLUS ANTHRACIS-SYMPTOMATICI AND ALLIED ORGANISMS WITH RESPECT TO GAS-PRODUCTION.*

By L. C. Todd.

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The question of the differentiation of the bacillus of symptomatic anthrax from that of malignant edema often arises. The fact that both possess the ability to produce gas when grown in carbohydrate-containing media, suggested that this characteristic might be used in differentiation. A study of the literature revealed such a paucity of data in this field that further work seemed necessary.

In this communication attention has been confined largely to the production of carbon dioxid and hydrogen and to their ratios. Work has also been done on the production of hydrogen sulfid and on several other biochemical characteristics, such as the production of methyl mercaptan, indol, and acids, and the action on nitrates. This work will be the substance of a later report.

The sources of the various strains are recorded in Table 1. Attention was paid especially to the two organisms mentioned, but several allied organisms were included for the sake of comparison. A very active gas-producing strain of B. coli was used as a control.

Sugar-free broth was prepared by allowing an active strain of B. coli to grow in regular beef infusion for 18 hours. The medium was then boiled 20 minutes and filtered, and 2% Witte's peptone and 0.5% sodium chlorid added. It was then titrated and adjusted to a reaction neutral to phenolphthalein.[1] The medium was sterilized fractionally for 30 minutes at 100 C. on each of 3 successive days.

Control inoculations in this sugar-free broth were made with a strain of B. coli which produced large quantities of gas in media containing dextrose, levulose, galactose, arabinose, mannose, rhamnose, xylose, lactose, maltose, saccharose, raffinose, sorbite, dulcite, mannite, and salicin; and also with a strain of B. mucosus-capsulatus that produced gas in media containing dextrose, levulose, galactose, mannose, lactose, maltose, saccharose, dextrin, and glycerin. In these controls no gas was produced during 10 days' incubation.

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* Received for publication October 18, 1916.

[1] Standard Methods of Water Analysis, 1912.

TABLE 1

The Sources of the Strains Studied

Organism and Strain No.		Source
B. anthracis-symptomatici	2	Blackleg vaccine from U. S. Dept. of Agriculture, Washington, D. C.
	3	An American commercial blackleg vaccine virus
	4	A tube of blackleg virus (desiccated blackleg meat) received from Dr. G. W. Dunphey, state veterinarian, Lansing, Mich.
	5	Culture from symptomatic anthrax, received from Dr. V. A. Moore, Cornell University, Ithaca, N. Y.
	6	A French commercial blackleg vaccine virus
	10	A French commercial blackleg vaccine virus
	12	An American commercial blackleg vaccine virus
	48	Culture received from an American commercial laboratory
	60	Culture received from Dr. F. G. Novy, University of Michigan, Ann Arbor
B. oedematis-maligni	421	Culture received from the culture bureau of the American Museum of Natural History, New York City
	485	Culture received from the culture bureau of the American Museum of Natural History, New York City
	558	Culture received from the culture bureau of the American Museum of Natural History, New York City
	560	Culture received from Dr. F. G. Novy
B. oedematis-maligni II	1	Culture received from Dr. Novy
B. botulinus	1	Culture received from Dr. Novy
Ghon-Sachs bacillus	1	Culture received from Dr. K. F. Meyer, University of California, Berkeley
B. coli (De Snoo)		Culture received from Dr. Poels, Holland state serum laboratory, Rotterdam

This broth was then used as the bearer of the various carbohydrates. These were added in 1% proportion and the resulting media, in 250-c.c. flasks, were heated 30 minutes in flowing steam, filled into sterile fermentation tubes, heated in the tubes for another 30 minutes in flowing steam, and then incubated 4 days to reveal contamination. It was found by this method that sterilization was accomplished with slight hydrolysis of the various carbohydrates.

Baseless fermentation tubes, holding 12 c.c. of media when filled to a convenient height, were used. These were readily handled in special racks holding 18 tubes each. They stood in such position that the closed arm was always upright and could not be jarred loose from this position during handling of the racks.

The following carbohydrates, alcohols, and glucosids[2] were used:

Monosaccharids—dextrose, levulose, galactose, arabinose, mannose, rhamnose, xylose.
Disaccharids—lactose, maltose, saccharose.
Trisaccharid—raffinose.
Polysaccharids—dextrin, inulin, glycogen, starch.
Alcohols—the trihydric, glycerin; the pentahydric, adonite; the hexahydrics, sorbite, dulcite, and mannite.
Glucosids—salicin, amygdalin, phlorizin.

The polysaccharids dissolved with some difficulty. Soluble starch, according to Lintner, was employed. The carbohydrates were of the best quality obtainable.

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[2] Hawk, Physiological Chemistry, 1914, p. 25. Eyre, Technique of Bacteriology, 1913, p. 178.

The experiment was made completely twice over, as indicated in Tables 2 and 3. In the first trial, as seed of the various strains, 4-day-old cultures in Martin's broth[3] were used. These strains had been carried as stock cultures in Martin's broth under hydrogen, the interval of transplant having been 6 days. In the second trial, the seed cultures had been trained for 2 generations in the plain sugar-free broth prepared. Otherwise, the conditions were maintained as nearly as possible the same in both experiments.

Inoculation of fermentation tubes was made by the pipet method, 4 drops of the sediment emulsion being used, which, in the case of the spore-bearers, contained enormous numbers of spores.

Readings were taken every 24 hours, and gas levels marked with water-proof hard-drying carbon ink. Readings were made for at least 11 consecutive days. Gas in the closed arm is indicated in the tables in percentage of total height of column. Less than 5% is designated — because amounts less than that can be estimated only approximately. Readings were made by means of a finely graduated gasometer at the back of the tube and to the bottom of the meniscus. Carbon dioxid was determined by absorption with 2% sodium-hydroxid solution.

In figuring out the so-called gas coefficients, the formula CO_2 H was used. This directs special attention to the carbon dioxid produced, since the resulting figure gives at a glance a comparative estimate of the carbon dioxid in proportion to the residual gas, which is largely hydrogen, though there is probably some methane in it also.[4] The greater the factor, the greater the proportionate amount of carbon dioxid produced.

For all practical purposes the coefficients obtained with gas of less than 10% mean little, unless there is a decided reduction in volume when the carbon dioxid is absorbed. Even a slight reduction—1%—in the amount of gas in the closed arm is detectable and measurable, but most emphasis should be placed on the coefficients obtained from gas produced in larger amounts.

During the sodium-hydroxid treatment, reduction of the gas volume present, in the small as well as in the larger amounts, indicates the presence of carbon dioxid and is of value as suggesting the splitting up of carbohydrate material. In this experiment in which so-called sugar-free broth was used as control on the sugar media, gas was produced by the majority of the strains. In no case was there even a suggestion of reduction of volume during the sodium-hydroxid treatment. There was no carbon

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[3] Besson, Bacteriology, Microbiology and Serum Therapy, 1913, p. [illegible].

[4] Besson, Bacteriology, Microbiology and Serum Therapy, 1913, p. 555.

TABLE 2

Comparison of Results in Gas-Production from Sugars by Bacillus Anthracis-Symptomatici and Allied Organisms *

Strain	Dextrose	Levulose	Galactose	Arabinose	Mannose	Xylose	Lactose	Maltose	Saccharose	Raffinose	Dextrin	Inulin
	Bacillus anthracis-symptomatici											
2	0 10	+ 22	+ 10	0 8	+ 10	+ 5	+ 12	0 20	0 10	0 5	0 10	25 30
	+ 10	7 22	5 10	+ 8	5 10	+ 5	+ 12	5 20	5 10	+ 5	+ 10	27 30
	+ 10	10 25	5 22	5 8	5 10	+ 5	5 12	10 20	7 10	5 7	5 10	30 30
	5 18	12 25	7 60	5 8	7 35	+ 5	7 12	12 20	10 10	5 7	7 10	30 80
	7 20	17 25	7 60	5 8	7 35	+ 5	7 12	15 28	10 10	5 7	10 10	30 30
	7	20	7	5	10	+ 5	10	20	10	5	10	30
	$x=\frac{10}{10}=1$	$x=\frac{17}{8}=2.12$	$x=\frac{5}{55}=0.09$	$x=\frac{3}{5}=0.6$	$x=\frac{8}{27}=0.29$	$x=\frac{2}{3}=0.67$	$x=\frac{0}{12}=0$	$x=\frac{20}{8}=2.5$	$x=\frac{3}{7}=0.4$	$x=\frac{2}{5}=0.4$	$x=\frac{0}{10}=0$	$x=\frac{5}{25}=0.2$
3	+ 15	0 30	0 5	0 10	0 7	+ 5	0 7	0 43	0 8	0 5	0 +	0 0
	+ 18	10 30	+ 5	+ 10	+ 7	+ 5	+ 7	7 50	+ 8	+ 5	0 +	0 0
	5 18	15 30	+ 57	8 10	5 7	+ 5	5 7	15 55	5 8	5 10	+ +	0 0
	7 20	18 30	5 60	8 10	7 7	+ 5	5 7	20 45	7 8	5 10	+ +	0 0
	10 23	25 30	5 60	8 10	7 7	5 5	5 7	25 62	7 8	5 10	+ +	0 0
	10	25	5	8	7	5	5	25	7	5	+	0
	$x=\frac{18}{5}=3.6$	$x=\frac{20}{10}=2$	$x=\frac{8}{52}=0.15$	$x=\frac{5}{5}=1$	$x=\frac{2}{5}=0.4$	$x=\frac{1}{4}=0.25$	$x=\frac{2}{5}=0.4$	$x=\frac{50}{12}=4.17$	$x=\frac{3}{5}=0.6$	$x=\frac{2}{8}=0.25$	$x=\frac{0}{+}=0$	$x=0$
4	0 7	+ 8	+ 7	0 7	0 8	+ 10	0 7	10 45	0 17	+ 12	0 8	+ 10
	+ 12	+ 8	+ 7	+ 7	+ 8	5 10	+ 7	10 48	+ 17	10 12	+ 8	5 10
	+ 12	+ 8	+ 25	5 7	5 8	7 10	+ 7	17 48	+ 17	12 12	+ 8	5 10
	+ 15	5 8	+ 27	5 10	7 8	7 10	+ 7	30 45	5 17	12 12	5 8	7 10
	+ 22	7 8	5 27	5 10	7 8	8 10	+ 7	35 52	5 17	12 12	5 8	7 10
	5	7	5	5	7	8	+	40	15	12	5	7
	$x=\frac{15}{7}=2.14$	$x=\frac{4}{4}=1.0$	$x=\frac{7}{20}=0.35$	$x=\frac{3}{7}=0.43$	$x=\frac{3}{5}=0.6$	$x=\frac{3}{7}=0.43$	$x=\frac{2}{5}=0.4$	$x=\frac{40}{12}=3.5$	$x=\frac{4}{13}=0.3$	$x=\frac{2}{10}=0.2$	$x=\frac{3}{5}=0.6$	$x=\frac{2}{8}=0.25$
5	0 40	0 42	+ 8	0 15	0 7	+ 8	0 10	0 48	0 25	0 8	0 5	+ 10
	+ 42	15 42	7 8	10 15	+ 7	5 8	5 10	20 50	5 25	+ 8	+ 5	5 10
	5 45	20 45	7 8	12 15	+ 7	7 8	7 10	30 50	7 25	5 8	+ 5	7 10
	7 45	30 45	7 10	15 15	+ 7	7 8	10 10	35 50	10 25	5 8	+ 5	7 10
	30 45	40 45	7 10	15 15	5 7	7 8	10 10	40 50	10 25	5 8	+ 5	7 10
	35	40	7	15	5	7	10	47	25	5	+	7
	$x=\frac{28}{17}=1.65$	$x=\frac{35}{10}=3.5$	$x=\frac{4}{6}=0.67$	$x=\frac{5}{10}=0.5$	$x=\frac{2}{5}=0.4$	$x=\frac{0}{8}=0$	$x=\frac{2}{8}=0.25$	$x=\frac{37}{13}=2.85$	$x=\frac{5}{20}=0.25$	$x=\frac{0}{8}=0$	$x=\frac{0}{5}=0$	$x=\frac{2}{8}=0.25$
6	0 70	0 22	+ 12	0 5	+ 20	0 8	0 8	0 25	0 7	0 5	0 8	0 7
	18 75	5 22	10 12	+ 5	15 20	+ 8	+ 8	5 25	+ 7	+ 5	+ 8	+ 7
	30 75	10 27	10 12	+ 5	17 20	+ 8	5 8	8 28	+ 7	+ 5	5 8	+ 20
	45 80	12 27	10 13	+ 7	17 20	+ 8	5 8	10 25	+ 7	+ 5	5 8	+ 20
	55 80	15 27	10 13	+ 7	18 20	5 8	5 8	15 35	+ 7	5 5	5 8	5 20
	65	20	10	5	20	5	5	20	5	5	7	5
	$x=\frac{60}{20}=3$	$x=\frac{19}{8}=2.37$	$x=\frac{6}{7}=0.84$	$x=\frac{2}{5}=0.4$	$x=\frac{7}{13}=0.53$	$x=\frac{2}{6}=0.33$	$x=\frac{1}{7}=0.14$	$x=\frac{25}{10}=2.5$	$x=\frac{2}{5}=0.4$	$x=\frac{0}{5}=0$	$x=\frac{3}{5}=0.6$	$x=\frac{5}{15}=0.33$
10	0 15	0 50	0 +	0 7	+ 10	0 7	0 10	0 20	0 10	0 5	0 35	+ 7
	+ 18	5 50	7 +	+ 7	5 10	+ 7	5 10	5 20	+ 10	+ 5	35 35	5 7
	+ 18	12 50	+ +	5 7	5 10	+ 7	7 10	8 25	7 10	5 5	35 35	7 63
	+ 100	45 50	+ +	5 7	7 10	+ 7	7 10	10 20	7 10	5 5	35 35	7 63
	+ 100	45 50	+ +	5 7	7 10	+ 7	7 10	15 20	7 10	5 5	35 35	7 63
	5	50	+	5	7	5	7	20	7	5	35	7
	$x=\frac{20}{80}=0.25$	$x=\frac{23}{27}=0.85$	$x=0$	$x=\frac{2}{5}=0.4$	$x=\frac{2}{8}=0.25$	$x=\frac{2}{5}=0.4$	$x=\frac{3}{7}=0.43$	$x=\frac{12}{8}=1.5$	$x=\frac{0}{10}=0$	$x=\frac{0}{5}=0$	$x=\frac{2}{33}=0.06$	$x=\frac{3}{60}=0.05$
12	0 5	0 24	0 8	0 10	0 7	+ 7	0 30	0 25	+ 18	0 5	0 0	0 15
	+ 5	10 24	+ 8	5 10	+ 7	+ 7	+ 30	7 25	5 18	+ 5	0 0	10 15
	+ 5	15 25	5 8	5 10	5 7	5 7	5 30	10 25	5 18	5 5	0 0	12 56
	+ 95	20 33	7 8	7 10	5 7	5 7	5 30	15 25	5 18	5 5	0 0	12 56
	+ 95	20 33	7 8	7 10	5 7	5 7	5 30	20 25	7 18	5 5	0 0	12 65
	+	20	7	7 10	8	5	5	25	15	5	0	12
	$x=\frac{10}{85}=0.12$	$x=\frac{18}{15}=1.2$	$x=\frac{3}{5}=0.6$	$x=\frac{3}{7}=0.43$	$x=\frac{2}{5}=0.4$	$x=\frac{1}{6}=0.14$	$x=\frac{5}{25}=0.2$	$x=\frac{15}{10}=1.5$	$x=\frac{3}{15}=0.2$	$x=\frac{0}{5}=0$	$x=0$	$x=\frac{15}{50}=0.3$

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* Readings made every 24 hours for 11 days. Amount of gas produced indicated in percentages. Less than 5% is designated +. $x = CO_2/H$.

Uninoculated control tubes of all media were negative throughout the 11 days' incubation.

TABLE 2.—*Continued*

COMPARISON OF RESULTS IN GAS PRODUCTION FROM SUGARS BY BACILLUS ANTHRACIS SYMPTOMATICI AND ALLIED ORGANISMS *

Glycogen	Starch	Glycerin	Adonite	Sorbite	Dulcite	Mannite	Rhamnose	Salicin	Amygdalin	Phlorizin	Carbohydrate-free Broth
Bacillus anthracis symptomatici											
0 5 + 5 + 5 + 5 5 5 5	0 5 0 5 + 5 5 5 5 5 5	0 8 + 8 + 10 + 42 5 42 5	+ 7 + 7 5 7 5 7 5 7 5	0 18 7 18 10 23 10 23 13 23 15	0 7 + 7 5 7 5 7 5 7 5	0 7 0 7 5 7 5 7 7 7 7	5 10 8 10 10 10 10 10 10 10 10	+ 25 15 27 17 27 20 27 22 27 25	+ 7 + 7 5 7 5 7 7 7 7	0 0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0 0
$x=\frac{0}{5}=0$	$x=\frac{0}{5}=0$	$x=\frac{2}{40}=0.05$	$x=\frac{1}{6}=0.16$	$x=\frac{10}{13}=0.77$	$x=\frac{2}{5}=0.4$	$x=\frac{1}{6}=0.16$	$x=\frac{1}{9}=0.11$	$x=\frac{7}{20}=0.34$	$x=\frac{2}{5}=0.4$	$x=0$	$x=0$
0 5 + 5 + 5 + 5 5 5 5	0 5 + 5 5 5 5 5 5 6 6	0 15 10 15 12 18 12 15 12 15 12	0 7 + 7 5 7 5 7 5 7 5	0 23 8 23 12 28 15 28 17 28 18	0 7 + 7 5 7 5 7 5 7 5	0 8 0 8 5 8 7 8 7 8 8	0 7 + 7 5 7 5 7 7 7 7	+ 32 20 35 25 35 30 35 30 35 32	+ 7 5 10 7 10 7 10 7 10 7	0 0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0 0
$x=\frac{0}{5}=0$	$x=\frac{0}{5}=0$	$x=\frac{3}{12}=0.25$	$x=\frac{1}{6}=0.16$	$x=\frac{13}{15}=0.87$	$x=\frac{1}{6}=0.16$	$x=\frac{0}{8}=0$	$x=\frac{0}{7}=0$	$x=\frac{11}{24}=0.46$	$x=\frac{0}{10}=0$	$x=0$	$x=0$
0 5 + 5 + 5 + 5 5 5 5	0 10 5 10 7 10 7 10 10 10 10	0 12 7 12 8 12 10 12 10 12 10	0 7 5 7 7 7 7 7 7 7 7	7 25 10 25 15 27 18 27 20 27 22	0 7 + 7 5 7 7 7 7 7 7	0 7 0 10 5 10 7 10 7 7	+ 5 + 5 5 5 5 5 5 5 5	0 80 15 32 20 32 25 32 25 32 30	0 6 + 5 5 5 5 6 5 6 5	0 0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0 0
$x=\frac{0}{5}=0$	$x=\frac{2}{8}=0.25$	$x=\frac{2}{10}=0.2$	$x=\frac{1}{6}=0.16$	$x=\frac{13}{14}=0.9$	$x=\frac{0}{7}=0$	$x=\frac{1}{9}=0.11$	$x=\frac{0}{5}=0$	$x=\frac{8}{24}=0.33$	$x=\frac{0}{5}=0$	$x=0$	$x=0$
+ 6 + 5 + 6 5 5 5 5 6	+ 8 5 10 7 10 7 10 8 10 8	0 8 + 8 5 8 7 8 7 8 7	+ 8 5 8 5 8 5 8 5 8 5	0 15 5 15 7 20 10 20 12 20 14	0 7 + 7 5 7 6 7 6 7 6	0 11 0 11 8 11 10 11 10 11 10	+ 5 + 5 5 5 5 5 5 5 5	+ 35 20 38 25 38 28 38 30 38 35	+ 8 + 8 7 8 7 8 7 8 7	0 0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0 0
$x=\frac{0}{6}=0$	$x=\frac{1}{9}=0.11$	$x=\frac{0}{8}=0$	$x=\frac{1}{7}=0.14$	$x=\frac{10}{10}=1$	$x=\frac{0}{7}=0$	$x=\frac{1}{10}=0.1$	$x=\frac{0}{5}=0$	$x=\frac{13}{25}=0.52$	$x=\frac{1}{7}=0.14$	$x=0$	$x=0$
+ 5 + 5 + 5 5 5 5 5 5	0 8 + 9 5 9 5 9 7 9 8	0 10 + 10 + 10 5 10 5 10 5	0 10 + 10 5 10 8 10 10 10 10	+ 48 12 48 13 70 18 70 22 70 30	0 7 + 7 5 7 6 7 6 7 6	+ 7 + 7 5 7 7 7 7 7 7	0 7 + 7 7 7 7 7 7 7 7	0 5 + 8 + 8 + 8 5 8 5	0 7 + 7 + 7 5 7 7 7 7	0 0 0 0 0 0 0 0 0 0 0	0 + 0 + 0 + 0 + 0 + +
$x=\frac{0}{5}=0$	$x=\frac{1}{8}=0.13$	$x=\frac{2}{8}=0.25$	$x=\frac{0}{10}=0$	$x=\frac{55}{15}=3.67$	$x=\frac{0}{7}=0$	$x=\frac{1}{6}=0.6$	$x=\frac{0}{7}=0$	$x=\frac{0}{8}=0$	$x=\frac{0}{7}=0$	$x=0$	$x=0$
0 5 + 5 5 5 5 5 5 5 5	0 7 + 8 + 8 5 8 7 8 7	0 17 10 17 15 17 15 17 15 17 15	+ 7 + 7 5 7 5 7 7 7 7	0 15 5 15 7 18 10 18 11 18 12	0 7 + 7 5 7 7 7 7 7 7	0 9 0 9 5 9 7 9 9 9 9	0 10 8 10 10 10 10 10 10 10 10	0 20 5 22 10 22 12 22 20 22 20	0 7 + 7 5 7 5 7 7 7 7	0 0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 + 0 + 0 +
$x=\frac{0}{5}=0$	$x=\frac{0}{8}=0$	$x=\frac{5}{12}=0.42$	$x=\frac{0}{7}=0$	$x=\frac{8}{10}=0.8$	$x=\frac{0}{7}=0$	$x=\frac{0}{9}=0$	$x=\frac{0}{10}=0$	$x=\frac{2}{20}=0.1$	$x=\frac{0}{7}=0$	$x=0$	$x=0$
0 5 + 5 + 5 + 5 5 5 5	0 7 + 8 5 8 5 8 7 8 7	0 8 + 8 + 8 + 8 5 8 5	0 8 + 8 5 8 5 8 5 8 5	+ 10 + 10 5 12 5 12 8 12 10	0 7 + 7 5 7 5 7 5 7 5	0 7 0 7 + 7 5 7 7 7 7	0 8 + 8 5 8 5 8 8 8 8	0 20 12 20 20 20 20 20 20 20 20	0 10 + 10 7 10 7 10 7 10 7	0 0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0 0
$x=\frac{0}{5}=0$	$x=\frac{0}{8}=0$	$x=\frac{2}{6}=0.33$	$x=\frac{2}{6}=0.33$	$x=\frac{3}{9}=0.25$	$x=\frac{1}{6}=0.16$	$x=\frac{0}{7}=0$	$x=\frac{0}{8}=0$	$x=\frac{0}{20}=0$	$x=\frac{0}{10}=0$	$x=0$	$x=0$

TABLE 2.—*Continued*

COMPARISON OF RESULTS IN GAS-PRODUCTION FROM SUGARS BY BACILLUS ANTHRACIS-SYMPTOMATICI AND ALLIED ORGANISMS *

Strain	Dextrose	Levulose	Galactose	Arabinose	Mannose	Xylose	Lactose	Maltose	Saccharose	Raffinose	Dextrin	Inulin
						Bacillus anthracis-symptomatici						
48	0 + + 5 + 5 + 7 + 7 + $x=\frac{0}{7}=0$	+ 35 13 35 15 40 20 55 27 55 30 $x=\frac{25}{30}=0.83$	0 5 + 5 + 5 + 13 5 13 5 $x=\frac{3}{10}=0.3$	0 8 + 8 5 8 5 8 5 8 5 $x=\frac{3}{5}=0.6$	0 12 + 12 10 12 12 12 12 12 12 $x=\frac{2}{10}=0.2$	+ 13 + 13 5 13 5 13 5 13 5 $x=\frac{8}{5}=1.6$	0 15 + 15 5 15 5 15 5 15 5 $x=\frac{0}{15}=0$	0 22 + 25 7 28 10 22 15 22 20 $x=\frac{17}{5}=3.4$	+ 15 13 15 15 15 15 13 15 13 15 $x=\frac{3}{10}=0.3$	+ 5 + 5 5 5 5 5 5 5 5 $x=\frac{0}{5}=0$	+ 5 + 5 + 5 + 5 + 5 + $x=\frac{0}{5}=0$	0 8 + 8 5 8 5 8 5 8 5 $x=\frac{0}{8}=0$
60	+ 47 12 47 23 50 30 50 38 50 42 $x=\frac{43}{7}=6.14$	5 52 12 52 20 55 30 55 38 55 42 $x=\frac{45}{10}=4.5$	5 10 5 10 8 10 8 10 10 10 10 $x=\frac{2}{8}=0.25$	+ 8 5 10 6 10 7 10 7 10 8 $x=\frac{2}{8}=0.25$	+ 7 + 7 5 7 7 7 7 7 7 $x=\frac{1}{6}=0.16$	+ 7 + 7 + 7 + 7 + 7 5 $x=\frac{1}{6}=0.16$	+ 5 + 5 5 5 5 5 5 5 5 $x=\frac{0}{5}=0$	+ 32 53 67 Broken	+ 8 + 8 7 8 8 8 8 8 8 $x=\frac{1}{7}=0.14$	+ 5 + 5 + 5 + 5 + 5 + $x=\frac{0}{5}=0$	0 + + + + + + 7 + 7 + $x=\frac{0}{7}=0$	+ 8 + 8 7 8 8 8 8 8 8 $x=\frac{0}{8}=0$
						Bacillus oedematis-maligni, I						
421	+ 25 10 25 15 25 20 25 20 25 20 $x=\frac{17}{8}=2.12$	+ 28 12 28 20 28 25 28 25 28 25 $x=\frac{18}{10}=1.8$	0 5 + 5 + 5 + 7 + 7 5 $x=\frac{2}{5}=0.4$	0 10 0 10 + 10 5 58 7 58 10 $x=\frac{3}{55}=0.05$	0 15 + 15 10 15 12 15 12 15 15 $x=\frac{3}{12}=0.25$	0 7 + 7 + 7 5 7 7 7 7 $x=\frac{0}{7}=0$	0 15 + 15 7 15 10 15 10 15 10 $x=\frac{3}{12}=0.25$	0 40 17 42 25 45 30 45 35 45 40 $x=\frac{30}{15}=2$	0 7 + 7 + 10 + 12 + 20 5 20 $x=\frac{5}{15}=0.33$	0 8 + 8 5 8 5 8 5 5 $x=\frac{0}{8}=0$	0 5 0 5 + 5 + 5 + 5 + $x=\frac{1}{4}=0.25$	+ 10 + 10 5 10 5 10 8 10 8 $x=\frac{5}{5}=1$
485	0 15 5 15 8 15 12 18 12 18 15 $x=\frac{12}{6}=2$	+ 45 17 45 30 50 35 50 40 50 45 $x=\frac{40}{10}=4$	0 7 + 7 5 7 5 7 5 7 5 $x=\frac{2}{5}=0.4$	0 5 0 5 + 5 5 8 5 8 5 $x=\frac{0}{8}=0$	0 12 5 12 10 12 10 12 10 12 10 $x=\frac{4}{8}=0.5$	+ 9 5 9 5 9 7 9 9 9 9 $x=\frac{0}{9}=0$	0 10 + 10 5 10 5 10 5 10 5 $x=\frac{0}{10}=0$	0 28 10 30 15 30 20 30 22 30 25 $x=\frac{20}{10}=2$	0 10 0 10 + 10 + 10 + 10 5 $x=\frac{0}{10}=0$	0 7 5 7 7 7 7 7 7 7 $x=\frac{0}{7}=0$	0 28 + 28 + 28 + 28 + 28 25 $x=\frac{4}{24}=0.17$	0 + + + + + + + + + + $x=\frac{0}{+}=0$
558	+ 14 5 15 5 17 10 20 10 20 10 $x=\frac{10}{10}=1$	0 17 + 17 5 18 10 22 12 22 15 $x=\frac{17}{5}=3.4$	0 + 0 + + + + + + 4 + $x=\frac{2}{2}=1$	0 10 5 10 5 10 5 10 7 10 7 $x=\frac{2}{8}=0.25$	0 10 5 10 5 10 10 10 10 10 10 $x=\frac{2}{8}=0.25$	+ 5 + 5 + 5 + 5 5 5 5 $x=\frac{1}{4}=0.25$	+ 5 + 7 + 10 5 13 5 13 5 $x=\frac{0}{13}=0$	+ 30 10 30 20 30 22 30 25 30 28 $x=\frac{20}{10}=2$	0 7 + 7 + 8 + 10 5 10 5 $x=\frac{0}{10}=0$	0 5 + 5 5 5 5 5 5 5 5 $x=\frac{0}{5}=0$	0 7 0 7 + 7 5 7 5 7 5 $x=\frac{2}{5}=0.4$	0 + 0 + + + + + + + + $x=\frac{0}{+}=0$
560	+ 8 5 8 5 8 7 8 8 8 8 $x=\frac{2}{6}=0.4$	0 24 5 28 8 30 12 33 15 33 20 $x=\frac{28}{5}=5.6$	5 17 12 17 15 17 15 17 15 17 16 $x=\frac{5}{12}=0.42$	0 + + + + + + + + + + $x=\frac{0}{+}=0$	+ 20 10 20 13 20 16 20 18 20 20 $x=\frac{5}{15}=0.33$	0 5 + 5 + 5 + 5 + 5 + $x=\frac{0}{5}=0$	0 8 + 8 + 8 7 8 7 8 7 $x=\frac{1}{7}=0.14$	0 20 5 20 10 20 13 20 16 20 18 $x=\frac{13}{7}=1.9$	7 18 10 18 15 18 15 18 15 18 17 $x=\frac{3}{15}=0.2$	0 5 + 5 + 5 5 5 5 5 5 $x=\frac{0}{5}=0$	+ + + 5 + 5 + 8 + 8 + $x=\frac{3}{5}=0.6$	0 5 + 5 + 5 + 5 + 5 + $x=\frac{0}{5}=0$

TABLE 2.—*Continued*

Comparison of Results in Gas-Production from Sugars by Bacillus Anthracis Symptomatici and Allied Organisms.*

Glycogen	Starch	Glycerin	Adonite	Sorbite	Dulcite	Mannite	Rhamnose	Salicin	Amygdalin	Phlorizin	Carbohydrate-free Broth
Bacillus anthracis-symptomatici											
0 5 + 5 5 5 5 5 5 5 5 $x=\frac{0}{5}=0$	0 13 8 15 12 15 12 15 13 15 13 $x=\frac{2}{13}=0.15$	0 8 + 8 5 8 5 8 5 8 5 $x=\frac{0}{8}=0$	+ 8 + 8 5 8 5 8 5 8 5 $x=\frac{0}{8}=0$	+ 20 12 20 13 23 15 23 16 23 20 $x=\frac{8}{15}=0.53$	0 7 + 7 5 7 5 7 5 7 5 $x=\frac{0}{7}=0$	0 10 0 10 5 10 7 10 10 10 10 $x=\frac{0}{10}=0$	+ 5 + 5 + 5 5 6 5 5 5 $x=\frac{0}{5}=0$	0 15 5 15 7 15 10 15 12 15 15 $x=\frac{3}{12}=0.25$	0 8 + 8 5 8 5 8 8 8 8 $x=\frac{0}{8}=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$
+ 5 + 5 + 5 + 5 + 5 5 $x=\frac{0}{5}=0$	0 7 + 8 5 8 5 8 7 8 7 $x=\frac{0}{8}=0$	+ 10 7 10 8 10 8 10 10 10 10 $x=\frac{2}{8}=0.25$	+ 5 5 5 5 6 5 5 5 5 5 $x=\frac{1}{4}=0.25$	+ 12 + 13 5 15 5 12 9 12 10 $x=\frac{4}{8}=0.5$	0 8 + 8 5 8 5 8 5 8 5 $x=\frac{0}{8}=0$	0 15 8 15 13 15 14 15 15 15 15 $x=\frac{5}{10}=0.5$	+ 5 + 5 + 5 5 5 5 5 5 $x=\frac{0}{5}=0$	+ 19 8 19 12 20 15 20 15 20 15 $x=\frac{4}{16}=0.25$	0 5 + 8 5 8 5 5 5 5 5 $x=\frac{0}{5}=0$	0 0 0 0 0 + 0 + 0 + 0 $x=\frac{0}{+}=0$	0 0 0 0 0 0 0 + 0 + 0 $x=0$
Bacillus oedematis-maligni, I											
0 + + + + + + + + 5 + $x=\frac{0}{5}=0$	0 8 + 8 + 8 5 8 6 8 8 $x=\frac{0}{8}=0$	0 17 0 17 + 17 5 17 12 17 15 $x=\frac{3}{14}=0.21$	0 10 + 10 7 10 7 10 8 10 10 $x=\frac{2}{8}=0.25$	15 30 20 30 23 32 23 32 27 32 28 $x=\frac{12}{20}=0.6$	0 9 + 9 + 9 5 9 5 9 5 $x=\frac{0}{9}=0$	0 10 0 12 + 12 7 12 10 12 10 $x=\frac{0}{12}=0$	0 15 + 15 12 15 12 15 12 15 15 $x=\frac{1}{14}=0.07$	0 12 0 15 0 20 + 20 5 20 10 $x=\frac{3}{17}=0.18$	0 5 + 8 + 8 5 8 5 8 5 $x=\frac{0}{8}=0$	0 0 0 0 0 + 0 + 0 + 0 $x=\frac{0}{+}=0$	0 + 0 + + + + + + + + $x=0$
0 + + + + + + + + 5 + $x=\frac{0}{5}=0$	+ 8 + 9 5 9 5 9 7 9 8 $x=\frac{2}{7}=0.28$	0 35 5 35 15 40 20 40 28 40 30 $x=\frac{18}{22}=0.82$	0 10 5 10 8 10 8 10 10 10 10 $x=\frac{0}{10}=0$	+ 10 + 10 5 13 5 10 8 10 10 $x=\frac{2}{8}=0.25$	0 10 5 10 7 10 8 10 8 10 8 $x=\frac{0}{10}=0$	0 8 0 8 5 8 5 8 7 8 7 $x=\frac{0}{8}=0$	+ 5 + 5 5 5 5 5 5 6 5 $x=\frac{0}{5}=0$	0 20 5 20 15 22 18 22 20 22 20 $x=\frac{2}{20}=0.1$	0 5 + 7 + 7 5 5 5 5 5 $x=\frac{0}{5}=0$	0 0 0 0 0 + 0 + 0 + 0 $x=\frac{0}{+}=0$	0 + 0 + 0 + + + + + + $x=0$
+ 5 + 5 + 5 + 5 + 5 + $x=\frac{0}{5}=0$	+ 13 10 13 12 13 12 13 13 13 13 $x=\frac{0}{13}=0$	0 10 + 10 + 18 5 18 5 18 5 $x=\frac{8}{10}=0.8$	+ 9 5 9 5 9 5 9 5 9 5 $x=\frac{0}{9}=0$	0 10 + 10 5 10 5 10 8 10 8 $x=\frac{0}{10}=0$	0 7 + 7 5 7 5 7 5 7 5 $x=\frac{0}{7}=0$	0 6 0 6 + 6 5 6 6 6 6 $x=\frac{0}{6}=0$	+ 5 + 5 + 5 + 6 5 5 5 $x=\frac{0}{5}=0$	0 27 10 27 20 27 25 27 27 27 27 $x=\frac{10}{17}=0.58$	+ 5 + 5 5 5 5 5 5 5 5 $x=\frac{0}{5}=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 0 0 + 0 + 0 + 0 + 0 $x=0$
0 + + + + + + + + + + $x=\frac{0}{3}=0$	0 8 + 8 5 8 5 8 8 8 8 $x=\frac{1}{7}=0.14$	0 8 + 8 + 10 + 10 + 10 8 $x=\frac{4}{6}=0.67$	0 5 + 5 5 7 5 7 5 5 $x=\frac{1}{6}=0.16$	0 8 + 10 5 12 5 12 7 12 8 $x=\frac{5}{7}=0.7$	+ 7 + 7 5 7 5 7 5 7 5 $x=\frac{0}{7}=0$	0 [illegible] 0 [illegible] + [illegible] [illegible] [illegible] [illegible] $x=\frac{0}{9}=0$	+ 5 + 5 + 10 5 5 [illegible] $x=\frac{0}{10}=0$	0 25 + 25 15 25 17 22 25 $x=\frac{5}{20}=0.25$	0 7 + 7 5 7 5 7 5 7 5 $x=\frac{0}{7}=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 [illegible] 0 [illegible] 0 [illegible] [illegible] 0 [illegible] 0 $x=$ [illegible]

TABLE 2.—*Continued*

COMPARISON OF RESULTS IN GAS-PRODUCTION FROM SUGARS BY BACILLUS ANTHRACIS-SYMPTOMATICI AND ALLIED ORGANISMS *

Dextrose	Levulose	Galactose	Arabinose	Mannose	Xylose	Lactose	Maltose	Saccharose	Raffinose	Dextrin	Inulin
Bacillus oedematis-maligni, II (Novy)											
+ 16	+ 28	+ 10	+ 8	+ 8	+ 5	+ 7	+ 32	+ 15	0 5	+ 5	+ 5
7 16	5 30	7 10	+ 8	5 8	+ 5	5 7	10 32	5 15	+ 6	+ 5	+ 6
12 16	10 30	8 10	7 10	7 12	+ 5	6 7	20 37	10 15	+ 5	+ 5	+ 5
14 16	17 32	8 10	7 10	7 48	+ 5	7 7	27 37	12 15	+ 5	5 5	5 8
15 16	23 32	10 10	8 10	8 48	+ 5	7 7	32 37	13 15	5 5	5 5	5 8
16	28	10	8	8	5	7	32	10	5	5	5
$x=\frac{11}{5}=2.2$	$x=\frac{24}{8}=3$	$x=\frac{2}{8}=0.25$	$x=\frac{2}{8}=0.25$	$x=\frac{8}{40}=0.2$	$x=\frac{2}{3}=0.67$	$x=\frac{0}{7}=0$	$x=\frac{27}{10}=2.7$	$x=\frac{5}{10}=0.5$	$x=\frac{0}{5}=0$	$x=\frac{0}{5}=0$	$x=\frac{0}{8}=0$
B. botulinus											
+ 5	0 11	0 +	0 5	0 5	0 5	7 12	+ 13	+ 10	0 5	+ 5	0 +
+ 5	+ 11	+ +	+ 5	+ 5	+ 5	7 12	5 13	+ 15	+ 5	+ 5	+ +
+ 7	5 11	+ +	+ 6	+ 5	+ 5	8 15	8 13	7 20	+ 5	+ 10	+ 5
+ 7	7 14	+ +	+ 5	+ 5	+ 5	10 15	10 13	8 28	5 5	+ 10	+ 5
+ 7	8 14	+ +	+ 5	5 5	+ 5	10 15	12 13	10 28	5 5	+ 10	+ 5
5	8	+	+	5	5	12	13	10	5	5	+
$x=\frac{2}{5}=0.4$	$x=\frac{9}{5}=1.8$	$x=\frac{0}{+}=0$	$x=\frac{1}{4}=0.25$	$x=\frac{1}{4}=0.25$	$x=\frac{2}{3}=0.67$	$x=\frac{2}{13}=0.15$	$x=\frac{10}{3}=3.33$	$x=\frac{16}{12}=1.33$	$x=\frac{0}{5}=0$	$x=\frac{0}{10}=0$	$x=\frac{0}{5}=0$
Ghon-Sachs bacillus											
0 65	0 10	0 25	0 10	0 0	0 8	0 20	0 12	0 7	0 0	0 12	0 8
0 65	0 10	0 25	+ 10	0 0	+ 8	0 20	0 12	0 7	0 0	0 12	0 8
0 65	? 13	0 30	5 10	0 0	5 8	7 20	? 15	0 32	0 0	0 12	0 8
15 63	+ 13	+ 30	5 100	0 15	5 8	10 20	+ 12	0 32	0 0	+ 12	+ 20
55 63	5 13	10 30	7 100	0 15	8 8	12 20	5 15	+ 32	0 0	10 12	5 20
65	7	17	7	0	8	15	10	+	0	10	5
$x=\frac{21}{42}=0.5$	$x=\frac{0}{13}=0$	$x=\frac{5}{25}=0.2$	$x=\frac{0}{100}=0$	$x=\frac{3}{12}=0.25$	$x=\frac{0}{8}=0$	$x=\frac{0}{20}=0$	$x=\frac{0}{15}=0$	$x=\frac{0}{32}=0$	$x=0$	$x=\frac{0}{12}=0$	$x=\frac{0}{20}=0$
B. coli (control)											
72 67	45 50	15 65	45 55	70 70	35 82	55 70	+ 80	30 63	50 87	0 32	0 0
75 67	55 50	50 65	55 55	75 70	52 85	68 70	40 80	53 63	70 87	0 32	0 0
75 67	58 50	60 65	60 55	78 70	60 85	70 70	70 80	65 63	78 87	+ 25	0 0
75 65	58 50	65 65	60 53	75 70	68 85	70 70	75 80	70 65	82 87	5 32	0 0
75 65	58 50	65 65	60 53	75 70	80 85	70 70	75 80	70 65	82 87	10 32	0 0
75	58	65	60	72	80	70	75	72	82	30	0
$x=\frac{15}{50}=0.3$	$x=\frac{15}{35}=0.43$	$x=\frac{20}{45}=0.44$	$x=\frac{13}{40}=0.33$	$x=\frac{23}{47}=0.5$	$x=\frac{45}{40}=1.13$	$x=\frac{25}{45}=0.55$	$x=\frac{30}{50}=0.6$	$x=\frac{20}{45}=0.44$	$x=\frac{44}{43}=1$	$x=\frac{2}{30}=0.07$	$x=0$

TABLE 2.—*Continued*

COMPARISON OF RESULTS IN GAS-PRODUCTION FROM SUGARS BY BACILLUS ANTHRACIS-SYMPTOMATICI AND ALLIED ORGANISMS *

Glycogen	Starch	Glycerin	Adonite	Sorbite	Dulcite	Mannite	Rhamnose	Salicin	Amygdalin	Phlorizin	Carbohydrate-free Broth
Bacillus oedematis-maligni, II (Novy)											
0 5 + 5 + 5 + 5 + 5 5 $x=\frac{0}{5}=0$	0 8 + 8 5 8 7 8 8 8 8 $x=\frac{1}{7}=0.14$	10 30 15 30 22 32 25 32 28 32 30 $x=\frac{15}{17}=0.9$	0 7 + 7 5 7 5 7 5 7 5 $x=\frac{0}{7}=0$	5 18 7 20 10 20 10 20 13 20 15 $x=\frac{10}{10}=1$	0 7 + 7 5 7 5 7 5 7 5 $x=\frac{0}{7}=0$	0 6 0 6 + 6 5 6 6 6 6 $x=\frac{0}{6}=0$	0 5 + 5 + 5 + 5 + 5 5 $x=\frac{0}{5}=0$	+ 12 6 12 7 12 8 12 10 12 10 $x=\frac{2}{10}=0.2$	+ 5 + 5 5 5 5 5 5 5 5 $x=\frac{0}{5}=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$
B. botulinus											
0 + + + + + + + + + + $x=\frac{0}{3}=0$	0 + + + + + + + + + + $x=\frac{0}{+}=0$	0 28 7 30 12 32 15 35 20 35 25 $x=\frac{18}{17}=1.06$	0 5 + 5 5 5 5 5 5 5 5 $x=\frac{2}{3}=0.67$	+ 18 5 21 7 22 10 22 12 22 15 $x=\frac{14}{8}=1\ 75$	0 6 + 6 5 6 5 6 5 6 5 $x=\frac{0}{6}=0$	0 5 0 5 + 5 + 5 5 5 5 $x=\frac{0}{5}=0$	0 5 + 5 + 5 + 5 + 5 5 $x=\frac{0}{5}=0$	+ 20 10 20 15 20 15 22 18 22 20 $x=\frac{5}{17}=0$	0 5 + 6 + 5 + 5 5 5 5 $x=\frac{0}{5}=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$
Ghon-Sachs baeillus											
+ 10 8 10 10 11 10 11 10 11 10 $x=\frac{0}{11}=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 8 0 15 0 17 0 17 + 17 5 $x=\frac{2}{15}=0.13$	0 8 0 8 0 8 0 8 + 8 8 $x=\frac{0}{8}=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 7 0 7 + 7 5 7 5 7 5 $x=\frac{0}{7}=0$	0 0 0 6 0 5 0 5 0 5 0 $x=\frac{0}{5}=0$	0 10 + 10 7 10 7 10 8 10 10 $=\frac{0}{10}=0$	0 7 0 10 + 37 5 37 5 37 7 $x=\frac{2}{35}=0.06$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$
B. coli (control)											
0 + + + + + + + + + + $x=\frac{0}{4}=0$	0 + + + + + + + + + + $x=\frac{0}{+}=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 5 + 5 + 5 + 5 5 5 5 $x=\frac{0}{5}=0$	62 83 82 80 90 80 92 70 88 70 83 $x=\frac{28}{42}=0.67$	0 11 + 11 5 12 8 12 10 12 10 $x=\frac{6}{6}=1$	37 70 52 70 60 75 65 76 70 70 70 $x=\frac{30}{40}=0.75$	27 57 47 60 50 60 55 60 57 60 57 $x=\frac{30}{30}=1$	0 55 + 55 47 55 55 55 55 55 55 $x=\frac{5}{50}=0\ 1$	0 5 + 8 + 8 + 8 5 8 5 $x=\frac{2}{6}=0.33$	0 + 0 + + + + + + 4 + $x=\frac{0}{4}=0$	Negative throughout

TABLE 3

Comparison of Results in Gas Production from Sugars by Bacillus Anthracis Symptomatici and Allied Organisms *

Strain	Dextrose	Levulose	Galactose	Arabinose	Mannose	Rhamnose	Xylose	Lactose	Maltose	Saccharose	Raffinose	Dextrin
	B. anthracis-symptomatici											
2	0 20 + 22 7 25 11 27 14 30 18 $x=\frac{15}{15}=1$	+ 62 25 65 40 66 50 69 55 69 60 $x=\frac{49}{20}=2.45$	0 + + + + + + + + + + $x=\frac{0}{+}=0$	0 5 + 5 + 5 + 5 + 5 + $x=\frac{1}{4}=0.25$	0 13 7 14 11 14 12 14 12 14 13 $x=\frac{4}{10}=0.4$	0 5 + 5 + 5 + 5 5 5 5 $x=\frac{0}{5}=0$	0 10 + 12 7 12 8 12 10 12 10 $x=\frac{4}{8}=0.5$	0 5 + 5 + 5 + 5 + 5 5 $x=\frac{0}{5}=0$	0 38 10 42 18 45 24 48 29 48 35 $x=\frac{36}{12}=3$	0 + + + + + + + + 5 + $x=\frac{0}{5}=0$	0 + 0 + + 5 + 5 + 5 + $x=\frac{0}{5}=0$	0 5 0 5 + 5 + 5 + 5 + $x=\frac{0}{5}=0$
3	0 46 11 50 22 51 32 52 37 52 43 $x=\frac{40}{12}=3.33$	+ 48 12 55 25 58 33 63 37 62 43 $x=\frac{50}{12}=4.17$	0 5 + 5 + 5 + 6 + 6 5 $x=\frac{1}{5}=0.2$	0 5 + 7 + 7 + 8 5 8 5 $x=\frac{4}{4}=1$	8 22 15 22 18 22 20 22 21 22 21 $x=\frac{8}{14}=0.57$	0 8 + 8 7 8 7 8 8 8 8 $x=\frac{0}{8}=0$	0 7 + 8 5 8 5 8 7 8 7 $x=\frac{2}{6}=0.33$	0 5 + 5 + 5 + 5 5 5 5 $x=\frac{0}{5}=0$	0 50 11 50 25 50 35 52 40 50 46 $x=\frac{37}{13}=3$	0 10 0 10 + 10 8 11 10 11 10 $x=\frac{2}{9}=0.22$	0 7 0 7 0 7 + 7 5 7 5 $x=\frac{0}{7}=0$	0 + 0 + 0 + + + + + + $x=\frac{0}{+}=0$
4	+ 60 15 60 30 60 40 60 50 60 57 $x=\frac{40}{20}=2$	+ 38 14 42 20 45 27 49 80 50 35 $x=\frac{36}{14}=2.57$	0 + + + + + + + + + + $x=\frac{0}{+}=0$	0 6 + 6 + 6 5 6 5 6 5 $x=\frac{1}{5}=0.2$	0 9 + 9 5 9 7 9 8 9 8 $x=\frac{4}{5}=0.8$	0 10 + 10 9 10 9 10 10 10 10 $x=\frac{1}{9}=0.11$	0 6 + 7 + 7 5 7 6 7 6 $x=\frac{2}{5}=0.4$	0 7 + 7 5 7 5 7 7 7 7 7 $x=\frac{1}{6}=0.17$	15 62 28 63 43 64 54 65 58 65 60 $x=\frac{43}{22}=2$	0 11 + 11 10 12 10 12 10 12 11 $x=\frac{2}{10}=0.2$	0 + + 5 + 5 + 5 + 5 + $x=\frac{1}{4}=0.25$	0 7 0 7 + 7 5 7 5 7 5 $x=\frac{0}{7}=0$
5	+ 32 15 32 22 33 27 33 50 33 31 $x=\frac{23}{10}=2.3$	+ 32 18 33 23 33 28 34 30 34 30 $x=\frac{21}{13}=1.61$	+ 16 13 17 15 17 15 17 15 17 15 $x=\frac{4}{13}=0.3$	0 5 + 5 + 5 + 5 + 5 5 $x=\frac{1}{4}=0.25$	0 10 + 10 7 10 8 10 8 10 8 $x=\frac{2}{8}=0.25$	0 8 + 9 7 9 7 9 8 9 8 $x=\frac{1}{8}=0.13$	0 7 + 8 + 8 5 8 7 8 7 $x=\frac{2}{6}=0.33$	0 7 + 7 5 7 5 7 7 7 7 $x=\frac{1}{6}=0.17$	0 45 20 45 30 45 37 47 40 46 42 $x=\frac{28}{18}=1.55$	0 15 0 15 10 15 13 15 13 15 13 $x=\frac{3}{12}=0.25$	+ 6 + 6 5 6 5 6 6 6 6 $x=\frac{1}{5}=0.2$	0 0 0 0 0 0 0 5 0 5 0 $x=\frac{0}{5}=0$
6	8 41 15 45 21 50 27 53 32 55 38 55 $x=\frac{42}{13}=3.2$	+ 75 17 80 30 80 47 76 60 76 70 $x=\frac{54}{22}=2.45$	+ 10 7 11 8 11 10 12 10 12 10 $x=\frac{4}{8}=0.5$	0 10 5 10 8 10 8 10 9 10 9 $x=\frac{1}{9}=0.11$	0 12 8 12 10 13 11 13 11 13 13 $x=\frac{3}{10}=0.3$	0 7 + 8 + 10 6 10 6 10 7 $x=\frac{0}{10}=0$	0 10 + 11 + 11 6 11 8 11 8 $x=\frac{5}{6}=0.83$	0 11 8 11 9 11 10 11 10 11 10 $x=\frac{1}{10}=0.1$	0 61 + 61 22 61 37 61 54 61 60 $x=\frac{44}{17}=2.6$	0 6 + 6 + 6 5 10 5 10 5 $x=\frac{3}{7}=0.42$	+ 8 + 8 7 8 7 8 8 8 8 $x=\frac{1}{7}=0.14$	0 5 + 5 + 5 + 5 + 5 5 $x=\frac{0}{5}=0$
10	0 28 10 30 15 33 20 34 25 35 35 $x=\frac{24}{11}=2.18$	0 48 11 52 25 57 37 59 42 63 63 $x=\frac{50}{13}=3.84$	+ 10 5 10 8 11 9 11 9 11 11 $x=\frac{4}{7}=0.56$	0 + + 7 + 7 + 7 + 7 7 $x=\frac{1}{6}=0.17$	0 8 + 9 7 9 8 9 8 9 9 $x=\frac{2}{7}=0.28$	0 10 + 10 7 10 9 10 9 10 10 $x=\frac{0}{10}=0$	0 60 38 62 47 62 52 62 56 62 60 $x=\frac{28}{34}=0.82$	0 8 + 8 7 8 8 8 8 8 8 $x=\frac{1}{7}=0.14$	+ 84 30 84 58 81 72 81 80 77 84 $x=\frac{55}{22}=2.5$	0 14 + 14 11 14 13 15 14 15 14 $x=\frac{3}{12}=0.25$	+ 7 + 7 6 7 6 7 7 7 7 $x=\frac{0}{7}=0$	0 5 0 5 + 5 + 5 + 5 + $x=\frac{0}{5}=0$
12	0 7 + 8 5 8 6 9 6 10 10 $x=\frac{5}{5}=1$	0 11 + 13 5 13 8 13 10 14 14 $x=\frac{8}{6}=1.25$	+ 7 5 8 6 8 7 8 7 8 8 $x=\frac{2}{6}=0.33$	0 5 + 5 + 5 + 5 + 5 5 $x=\frac{2}{3}=0.67$	0 11 7 12 10 12 11 12 11 12 12 $x=\frac{3}{9}=0.33$	0 10 + 10 7 10 8 10 8 10 10 $x=\frac{2}{8}=0.25$	0 8 + 8 + 9 7 9 7 9 8 $x=\frac{2}{7}=0.28$	0 8 + 8 7 8 8 8 8 8 8 $x=\frac{0}{8}=0$	10 55 37 55 46 55 53 55 55 51 55 $x=\frac{33}{18}=1.83$	0 10 0 11 0 11 0 11 10 11 10 11 $x=\frac{0}{11}=0$	+ 10 8 10 10 10 10 10 10 10 10 $x=\frac{1}{9}=0.11$	0 10 0 10 + 10 8 10 10 10 10 $x=\frac{1}{9}=0.11$

* Readings made every 24 hours for 11 days. Amount of gas produced indicated in percentages. Less than 5% is designated +. x = CO_2/H.
Blank control negative throughout.

TABLE 3.—*Continued*

COMPARISON OF RESULTS IN GAS-PRODUCTION FROM SUGARS BY BACILLUS ANTHRACIS-SYMPTOMATICI AND ALLIED ORGANISMS *

Inulin	Glycogen	Starch	Glycerin	Adonite	Sorbite	Dulcite	Mannite	Salicin	Amygdalin	Phlorizin	Plain Carbohydrate-free Broth
B. anthracis symptomatici											
0 5 0 5 + 5 + 5 + 5 5 $x=\frac{0}{5}=0$	0 0 0 0 0 0 0 0 0 0 0 $x=\frac{0}{+}=0$	0 6 0 6 0 6 + 6 + 6 6 $x=\frac{0}{6}=0$	0 10 0 10 + 10 9 10 9 10 10 $x=\frac{0}{10}=0$	0 5 0 5 0 5 + 5 + 5 5 $x=\frac{0}{5}=0$	0 30 56 0 37 11 42 16 47 19 50 25 53 $x=\frac{35}{21}=1.67$	0 5 0 7 0 7 + 7 + 7 5 $x=\frac{0}{7}=0$	0 14 0 15 10 16 12 15 13 15 14 $x=\frac{2}{13}=0.15$	0 8 13 0 10 0 10 + 10 6 11 8 12 $x=\frac{3}{10}=0.3$	0 9 0 10 0 10 + 10 7 10 9 $x=\frac{0}{10}=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 10 0 10 7 10 9 10 10 10 10 $x=\frac{0}{10}=0$
0 + 0 + 0 + + + + + + $x=\frac{0}{+}=0$	0 + 0 + + 5 + 5 + 5 + $x=\frac{0}{5}=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 8 0 8 + 8 7 8 7 8 8 $x=\frac{0}{8}=0$	0 8 0 8 0 8 + 9 6 9 8 $x=\frac{0}{9}=0$	0 11 15 + 13 7 13 8 14 10 15 11 15 $x=\frac{4}{11}=0.36$	0 7 0 7 + 7 5 7 7 7 7 $x=\frac{0}{7}=0$	0 11 0 11 + 12 9 12 10 12 10 $x=\frac{2}{10}=0.2$	0 + 12 0 6 0 8 0 8 0 10 + 12 $x=\frac{2}{10}=0.2$	0 5 0 7 0 7 + 7 + 7 5 $x=\frac{0}{7}=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 7 0 9 0 9 0 9 0 9 + $x=\frac{0}{9}=0$
0 + + + + + + + + + + $x=\frac{0}{+}=0$	0 5 + 5 + 5 + 5 + 5 + $x=\frac{2}{3}=0.4$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 11 0 11 + 11 9 11 10 11 11 $x=\frac{0}{11}=0$	0 10 + 10 7 10 8 11 8 11 10 $x=\frac{3}{8}=0.38$	0 14 18 0 16 7 16 10 17 11 18 14 18 $x=\frac{4}{14}=0.29$	0 10 0 12 7 12 9 12 10 12 10 $x=\frac{2}{10}=0.2$	0 13 + 13 10 13 11 13 12 13 12 $x=\frac{2}{11}=0.18$	0 13 22 0 14 0 15 0 17 + 20 12 22 $x=\frac{4}{18}=0.22$	0 5 0 5 0 5 + 5 + 5 + $x=\frac{0}{5}=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 10 0 10 0 10 + 10 6 10 10 $x=\frac{0}{10}=0$
0 + 0 5 + 5 + 5 + 5 + $x=\frac{0}{5}=0$	0 0 0 + 0 + 0 + 0 0 $x=\frac{0}{+}=0$	0 8 + 8 7 8 7 8 7 8 8 $x=\frac{0}{8}=0$	0 13 0 13 0 13 12 13 13 13 $x=\frac{0}{13}=0$	0 12 + 12 10 12 10 12 10 12 12 $x=\frac{1}{11}=0.09$	0 12 14 + 12 7 12 9 12 9 13 12 14 $x=\frac{4}{10}=0.4$	0 7 0 7 + 7 5 7 7 7 7 $x=\frac{0}{7}=0$	0 15 0 15 7 15 13 15 14 15 14 $x=\frac{1}{14}=0.07$	0 0 13 0 5 0 5 0 11 0 12 0 13 $x=\frac{0}{13}=0$	0 7 0 8 0 8 + 8 + 8 7 $x=\frac{0}{8}=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 0 0 7 0 10 0 10 0 10 0 $x=\frac{0}{10}=0$
0 + + + + + + + + + + $x=\frac{0}{+}=0$	0 7 + 7 5 7 6 7 7 7 7 $x=\frac{1}{6}=0.17$	0 17 + 17 14 17 14 17 15 17 17 $x=\frac{2}{15}=0.13$	0 9 0 9 0 9 0 9 0 9 0 $x=\frac{0}{9}=0$	0 8 0 8 0 8 5 8 7 8 8 $x=\frac{1}{7}=0.14$	0 20 33 0 23 10 25 15 28 16 31 18 32 $x=\frac{13}{20}=0.65$	0 8 0 10 7 10 7 10 8 10 8 $x=\frac{1}{9}=0.11$	0 11 0 11 0 12 0 12 6 12 11 $x=\frac{0}{12}=0$	0 0 5 0 0 0 0 0 + 0 + 0 5 $x=\frac{0}{5}=0$	0 + 0 5 0 5 + 5 + 5 + $x=\frac{0}{6}=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	+ 14 8 14 13 14 14 14 14 14 14 $x=\frac{0}{14}=0$
0 + 0 + + + + + + + + $x=\frac{0}{+}=0$	0 + 0 + + + + + + + + 5 $x=\frac{0}{5}=0$	0 8 + 8 + 8 7 8 8 8 8 $x=\frac{1}{7}=0.14$	0 10 0 11 0 11 0 11 0 11 8 $x=\frac{0}{11}=0$	0 6 0 6 + 6 + 6 5 6 6 $x=\frac{0}{6}=0$	0 25 35 0 30 12 30 15 32 18 33 23 34 $x=\frac{14}{21}=0.67$	0 9 0 10 7 10 8 10 9 10 9 $x=\frac{1}{9}=0.11$	10 76 85 76 83 75 78 72 75 72 78 $x=\frac{19}{53}=0.36$	0 35 42 0 38 30 40 33 40 33 41 35 42 $x=\frac{18}{23}=0.82$	0 + 0 + 0 + + 5 + 5 + $x=\frac{0}{5}=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 13 0 13 + 13 8 13 11 13 13 $x=\frac{0}{13}=0$
0 0 0 + 0 + 0 + 0 + 0 $x=\frac{0}{+}=0$	0 + 0 + 0 + 0 + 0 + + $x=\frac{0}{+}=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 8 0 9 0 9 0 9 8 9 8 $x=\frac{0}{9}=0$	0 11 0 11 8 11 8 11 10 11 11 $x=\frac{1}{10}=0.1$	0 25 31 0 28 15 28 17 30 19 31 23 31 $x=\frac{9}{22}=0.4$	0 5 0 6 + 6 + 6 5 6 5 $x=\frac{0}{6}=0$	0 8 0 8 + 8 7 8 8 8 8 $x=\frac{0}{8}=0$	0 23 27 0 25 0 25 + 25 13 26 20 27 $x=\frac{4}{23}=0.17$	0 6 0 6 0 6 + 6 + 6 + $x=\frac{0}{6}=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$

TABLE 3. —*Continued*

COMPARISON OF RESULTS IN GAS-PRODUCTION FROM SUGARS BY BACILLUS ANTHRACIS-SYMPTOMATICI AND ALLIED ORGANISMS *

Strain	Dextrose	Levulose	Galactose	Arabinose	Mannose	Rhamnose	Xylose	Lactose	Maltose	Saccharose	Raffinose	Dextrin
						B. anthracis-symptomatici						
48	+ 28 7 32 11 35 15 40 18 41 25 41 $x=\frac{32}{9}=3.55$	0 24 + 26 8 26 14 28 18 28 21 $x=\frac{20}{8}=2.5$	+ 9 5 9 6 10 8 10 8 10 10 $x=\frac{1}{9}=0.11$	0 5 + 5 + 5 + 5 + 5 5 5 $x=\frac{2}{3}=0.67$	0 10 + 10 5 12 8 12 8 12 9 $x=\frac{3}{9}=0.33$	0 7 + 7 5 7 7 7 7 7 7 $x=\frac{0}{7}=0$	0 19 10 20 13 20 15 20 17 20 19 $x=\frac{7}{13}=0.53$	0 5 + 5 + 5 5 5 5 5 5 $x=\frac{0}{5}=0$	+ 43 10 47 15 50 23 54 28 54 37 $x=\frac{41}{13}=3.15$	0 8 + 8 6 8 6 8 6 8 6 $x=\frac{1}{7}=0.14$	0 11 0 11 0 11 0 11 0 11 10 $x=\frac{1}{10}=0.1$	0 8 + 8 7 8 7 8 7 8 7 $x=\frac{1}{7}=0.14$
60	+ 43 8 48 13 52 18 53 25 53 35 $x=\frac{43}{10}=4.3$	10 74 18 75 28 73 43 73 53 70 67 $x=\frac{58}{12}=4.8$	+ 8 5 8 6 8 7 8 7 8 8 $x=\frac{3}{5}=0.6$	0 + + + + + + + + 5 + $x=\frac{1}{4}=0.25$	0 7 + 7 + 7 5 7 5 7 6 $x=\frac{1}{6}=0.17$	0 5 + 6 + 6 5 6 5 6 5 $x=\frac{1}{5}=0.2$	0 10 + 10 5 10 7 10 9 10 9 $x=\frac{3}{7}=0.42$	0 5 + 7 + 7 + 7 + 7 + $x=\frac{0}{7}=0$	5 75 14 77 35 77 54 77 65 75 72 $x=\frac{57}{18}=3.16$	0 11 0 11 8 11 10 11 10 11 11 $x=\frac{2}{9}=0.22$	+ 5 + 5 + 5 + 5 + 5 + $x=\frac{1}{4}=0.25$	0 + 0 + + + + + + + + $x=\frac{0}{+}=0$
						Ghon-Sachs bacillus I						
	0 12 0 17 + 20 + 23 7 24 24 $x=\frac{8}{16}=0.5$	0 + 0 5 0 7 + 10 + 10 + $x=\frac{3}{7}=0.42$	0 15 0 18 0 21 + 25 7 28 12 $x=\frac{8}{20}=0.4$	0 5 0 5 0 5 + 6 + 6 5 $x=\frac{0}{6}=0$	0 0 0 0 0 0 0 0 0 0 .... x = — = 0	0 33 0 33 25 33 30 32 32 32 33 $x=\frac{12}{20}=0.6$	0 + 0 + 0 + 0 + 0 + 0 $x=\frac{0}{+}=0$	0 0 0 0 0 9 0 13 0 15 0 $x=\frac{2}{13}=0.15$	0 20 + 20 5 20 8 24 13 24 16 $x=\frac{6}{18}=0.33$	0 8 0 8 + 8 + 8 7 8 7 $x=\frac{0}{8}=0$	0 10 0 10 + 10 7 10 8 10 8 $x=\frac{0}{10}=0$	0 + 0 + 0 5 0 5 0 5 + $x=\frac{0}{5}=0$
						B. oedematis maligni, I						
421	10 28 15 30 18 30 21 31 24 31 25 $x=\frac{21}{10}=2.1$	0 14 + 15 8 15 11 15 12 15 13 $x=\frac{10}{5}=2$	+ 10 6 10 7 10 8 10 8 10 8 $x=\frac{3}{7}=0.42$	0 5 + 5 + 5 + 5 + 5 + $x=\frac{1}{4}=0.25$	+ 13 + 13 7 13 9 13 10 13 12 $x=\frac{4}{9}=0.44$	0 7 + 7 + 7 5 7 7 7 7 $x=\frac{1}{6}=0.17$	0 10 + 10 + 11 6 11 7 11 7 $x=\frac{4}{7}=0.57$	+ 7 5 7 6 7 6 7 6 7 6 $x=\frac{1}{6}=0.17$	35 71 60 71 70 69 75 69 73 65 71 $x=\frac{13}{52}=0.25$	0 13 + 14 9 14 10 15 12 15 13 $x=\frac{4}{11}=0.36$	+ 8 5 8 7 8 7 8 7 8 7 $x=\frac{1}{7}=0.14$	0 5 + 5 + 5 + 5 5 5 5 $x=\frac{0}{5}=0$
485	+ 22 12 23 15 23 17 24 18 24 20 $x=\frac{14}{10}=1.4$	0 19 + 20 10 20 14 20 16 20 18 $x=\frac{11}{9}=1.2$	+ 10 7 10 8 11 10 11 10 11 10 $x=\frac{2}{9}=0.22$	0 8 + 8 5 8 7 8 7 8 7 $x=\frac{1}{7}=0.14$	0 13 8 13 10 13 12 13 12 13 13 $x=\frac{2}{11}=0.18$	0 11 7 11 9 11 9 11 10 11 11 $x=\frac{1}{10}=0.1$	0 13 7 13 8 13 10 13 12 13 12 $x=\frac{4}{9}=0.44$	+ 10 8 10 9 10 10 10 10 10 10 $x=\frac{0}{10}=0$	+ 55 35 55 48 55 57 55 56 50 56 $x=\frac{33}{17}=1.94$	0 13 + 13 9 13 10 13 11 13 11 $x=\frac{3}{10}=0.3$	0 14 0 14 0 14 8 15 12 15 13 $x=\frac{2}{13}=0.15$	0 + 0 + + + + + + + + $x=\frac{0}{+}=0$
558	+ 17 8 18 10 20 13 20 14 20 15 $x=\frac{12}{8}=1.5$	0 12 + 13 5 13 8 14 9 14 10 $x=\frac{9}{5}=1.8$	0 5 + 5 + 6 5 6 5 6 5 $x=\frac{0}{6}=0$	0 + + 5 + 5 + 5 + 5 + $x=\frac{0}{5}=0$	0 15 11 15 13 15 14 15 14 15 15 $x=\frac{3}{12}=0.25$	+ 12 10 12 10 12 11 12 11 12 11 $x=\frac{0}{12}=0$	0 7 + 8 + 8 6 8 7 8 7 $x=\frac{2}{6}=0\ 33$	0 8 5 8 6 8 7 8 7 8 7 $x=\frac{1}{7}=0.14$	13 72 42 71 58 71 68 71 70 69 70 $x=\frac{51}{18}=2.83$	0 12 + 13 10 13 10 13 10 13 12 $x=\frac{2}{11}=0.18$	+ 10 + 10 + 10 10 10 10 10 10 $x=\frac{0}{10}=0$	+ 6 5 6 5 6 5 6 6 6 6 $x=\frac{0}{6}=0$

TABLE 3.—*Continued*

Comparison of Results in Gas-Production from Sugars by Bacillus Anthracis-Symptomatici and Allied Organisms *

Inulin	Glycogen	Starch	Glycerin	Adonite	Sorbite	Dulcite	Mannite	Salicin	Amygdalin	Phlorizin	Plain Carbohydrate-free Broth
B. anthracis-symptomatici											
0 6 + 7 6 7 6 7 6 7 6 $x=\frac{0}{7}=0$	0 8 0 8 0 8 5 8 7 8 7 $x=\frac{4}{4}=1$	0 8 0 8 + 8 7 8 8 8 8 $x=\frac{0}{8}=0$	0 8 0 8 0 8 + 8 7 8 8 $x=\frac{0}{8}=0$	+ 6 + 6 5 6 5 6 5 6 6 $x=\frac{0}{6}=0$	+ 43 68 12 51 14 57 15 62 18 64 35 66 $x=\frac{47}{21}=2.24$	0 9 0 10 8 10 8 10 9 10 9 $x=\frac{1}{9}=0.11$	0 8 + 8 + 8 7 8 7 8 7 $x=\frac{0}{8}=0$	0 17 25 0 20 0 21 + 21 10 23 16 25 $x=\frac{5}{20}=0.25$	0 10 0 12 0 12 6 12 9 12 10 $x=\frac{0}{12}=0$	0 + 0 + 0 + 0 + + + + $x=\frac{0}{+}=0$	0 10 0 12 0 12 0 12 8 12 10 $x=\frac{0}{12}=0$
0 4 0 4 + 4 + 4 + 4 + $x=\frac{0}{4}=0$	0 + 0 + 0 + 0 + 0 + + $x=\frac{0}{+}=0$	0 7 0 8 + 8 5 8 7 8 7 $x=\frac{0}{8}=0$	0 10 0 10 0 10 + 10 9 10 10 $x=\frac{0}{10}=0$	0 + 0 + + 6 + 6 + 6 + $x=\frac{0}{6}=0$	0 18 26 0 20 10 22 12 23 13 25 16 26 $x=\frac{8}{18}=0.44$	0 6 0 6 + 6 + 6 + 6 6 $x=\frac{0}{6}=0$	0 10 0 12 + 12 10 12 10 12 10 $x=\frac{1}{11}=0.09$	0 0 5 0 0 0 0 0 0 0 + 0 5 $x=\frac{0}{5}=0$	0 5 0 5 0 7 + 7 + 7 5 7 $x=\frac{0}{7}=0$	0 0 0 0 0 + 0 + 0 + 0 $x=\frac{0}{+}=0$	0 7 0 7 0 7 + 7 5 7 5 $x=\frac{0}{7}=0$
Ghon-Sachs bacillus I											
0 9 0 10 + 10 7 10 7 10 9 $x=\frac{0}{10}=0$	0 10 0 10 0 10 + 10 9 10 9 $x=\frac{0}{10}=0$	0 + 0 + 0 + + + + + + $x=\frac{0}{+}=0$	0 + 0 + 0 + 0 + + + + $x=\frac{0}{+}=0$	0 12 0 12 0 12 + 12 11 12 12 $x=\frac{0}{12}=0$	0 0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 92 70 92 77 92 82 87 85 87 92 $x=\frac{27}{60}=0.45$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 0 0 + 0 + 0 + 0 + 0 $x=\frac{0}{+}=0$	0 0 0 0 0 + 0 + 0 + 0 $x=\frac{0}{+}=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$
B. oedematis-maligni, I											
0 + 0 5 0 5 + 5 + 5 + $x=\frac{0}{5}=0$	0 7 + 7 + 7 + 7 5 7 5 $x=\frac{1}{6}=0.17$	0 17 15 17 17 17 17 17 17 17 17 $x=\frac{1}{16}=0.06$	0 + 0 8 0 8 0 8 0 8 0 $x=\frac{0}{8}=0$	0 11 0 11 7 11 9 11 9 11 11 $x=\frac{1}{10}=0.1$	0 12 24 0 15 0 16 0 18 6 20 11 22 $x=\frac{6}{18}=0.33$	0 16 0 17 8 17 13 17 14 17 16 $x=\frac{2}{15}=0.13$	0 7 0 8 0 8 0 8 + 8 + $x=\frac{0}{8}=0$	0 11 19 0 13 0 15 0 15 + 17 9 19 $x=\frac{0}{19}=0$	0 5 0 6 0 6 + 6 + 6 + $x=\frac{0}{6}=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 15 0 15 0 15 + 15 13 15 15 $x=\frac{0}{15}=0$
0 5 0 7 5 7 5 7 5 7 5 $x=\frac{0}{7}=0$	0 5 0 5 0 5 0 5 0 5 + $x=\frac{1}{4}=0.25$	0 5 0 5 0 5 0 5 + 5 5 $x=\frac{0}{5}=0$	0 25 0 28 0 28 14 28 20 27 24 $x=\frac{11}{16}=0.69$	0 8 + 8 7 8 7 8 7 8 8 $x=\frac{1}{7}=0.14$	0 43 60 0 51 18 55 23 57 25 59 33 60 $x=\frac{32}{28}=1.14$	+ 11 10 12 10 12 10 12 10 12 11 $x=\frac{2}{10}=0.2$	0 10 + 12 + 12 8 12 9 12 10 $x=\frac{2}{10}=0.2$	0 19 31 0 23 0 25 + 25 9 27 15 29 $x=\frac{3}{28}=0.11$	0 11 0 11 + 12 8 12 10 12 11 $x=\frac{0}{12}=0$	0 0 0 0 0 + 0 + 0 + 0 $x=\frac{0}{+}=0$	0 10 0 11 0 11 8 11 10 11 10 $x=\frac{0}{11}=0$
0 + 0 + + + + + + + + $x=\frac{0}{+}=0$	0 + 0 + 0 + + 7 + 7 + $x=\frac{1}{6}=0.17$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 8 0 9 + 9 6 9 6 9 8 $x=\frac{0}{9}=0$	0 + 0 5 0 5 0 5 0 5 + $x=\frac{0}{5}=0$	8 21 26 18 22 19 23 19 23 19 25 20 26 $x=\frac{8}{18}=0.44$	0 11 + 11 10 11 10 11 10 11 11 $x=\frac{1}{10}=0.1$	0 7 0 8 + 8 + 8 5 8 7 $x=\frac{0}{8}=0$	0 30 33 + 32 12 32 25 32 26 33 29 33 $x=\frac{8}{25}=0.32$	0 10 0 10 0 10 + 10 8 10 8 $x=\frac{0}{10}=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 13 0 13 8 13 13 13 13 13 13 $x=\frac{0}{13}=0$

TABLE 3.—*Continued*

COMPARISON OF RESULTS IN GAS-PRODUCTION FROM SUGARS BY BACILLUS ANTHRACIS SYMPTOMATICI AND ALLIED ORGANISMS *

Strain	Dextrose	Levulose	Galactose	Arabinose	Mannose	Rhamnose	Xylose	Lactose	Maltose	Saccharose	Raffinose	Dextrin
B. oedematis-maligni, I												
560	0 7 + 7 + 8 5 8 5 8 6 $x=\frac{5}{3}=1.67$	0 17 + 18 5 18 10 20 13 20 15 $x=\frac{15}{5}=3$	0 + + + + + + + + + + $x=\frac{0}{+}=0$	0 + + + + 4 + 4 + 4 + $x=\frac{1}{3}=0.33$	0 11 + 13 5 13 7 14 8 14 10 $x=\frac{4}{10}=0.4$	0 8 + 8 + 8 7 8 8 8 8 $x=\frac{0}{8}=0$	0 7 + 7 + 7 + 7 5 7 5 $x=\frac{1}{6}=0.17$	0 10 7 10 10 10 10 13 10 13 10 $x=\frac{\text{[illegible]}}{10}=0.3$	+ 18 10 18 13 19 15 19 15 19 17 $x=\frac{14}{5}=2.8$	0 23 + 24 10 25 13 28 17 28 20 $x=\frac{10}{18}=0.55$	+ 10 + 10 9 10 10 10 10 10 10 $x=\frac{2}{8}=0.25$	0 + + + + + + + + + + $x=\frac{0}{+}=0$
B. oedematis-maligni, II, (Novy 1-ME II)												
	0 15 + 16 8 16 11 16 13 16 15 $x=\frac{12}{4}=3$	0 12 + 14 + 15 7 16 7 16 10 $x=\frac{9}{7}=1.3$	0 8 + 8 6 8 7 8 7 8 7 $x=\frac{2}{6}=0.33$	0 5 + 5 + 5 + 5 5 5 5 $x=\frac{2}{3}=0.67$	0 0 0 4 0 4 0 4 0' 4 0 $x=\frac{1}{3}=0.33$	0 8 0 8 + 8 + 8 7 8 8 $x=\frac{0}{8}=0$	0 6 0 8 + 8 5 8 6 8 6 $x=\frac{2}{6}=0.33$	0 6 + 6 5 6 5 6 6 6 6 $x=\frac{0}{6}=0$	0 26 8 26 15 27 20 27 23 27 25 $x=\frac{21}{6}=3.5$	0 + + 5 + 5 + 5 + 5 + $x=\frac{1}{4}=0.25$	0 6 + 6 + 6 + 6 + 6 + $x=\frac{1}{5}=0.2$	0 5 0 5 + 5 + 5 + 5 5 $x=\frac{0}{5}=0$
B. botulinus												
	0 13 5 14 8 14 10 14 10 14 12 $x=\frac{9}{5}=1\ 8$	0 12 + 14 + 14 5 15 8 15 10 $x=\frac{10}{5}=2$	0 5 + 5 + 5 + 5 5 5 5 $x=\frac{1}{4}\ 0.25$	0 5 + 5 + 5 + 6 + 6 5 $x=\frac{0}{6}=0$	0 6 + 6 + 6 5 6 5 6 5 $x=\frac{0}{6}=0$	0 5 + 5 + 5 + 5 + 5 5 $x=\frac{0}{5}=0$	0 8 + 8 + 8 5 8 7 8 7 $x=\frac{2}{6}=0.33$	0 7 + 7 + 7 5 7 5 7 5 $x=\frac{1}{6}=0.17$	0 15 + 17 8 19 10 19 12 19 13 $x=\frac{11}{8}=1.37$	0 10 + 10 6 10 7 10 8 10 8 $x=\frac{2}{8}=0.25$	+ 5 + 5 5 6 5 5 5 5 5 $x=\frac{0}{5}=0$	0 7 0 7 + 7 + 7 6 7 6 $x=\frac{0}{7}=0$
B. coli (control)												
	33 55 48 56 51 58 55 60 55 63 55 $x=\frac{30}{33}=0.90$	67 70 70 71 71 71 73 72 70 72 70 $x=\frac{30}{42}=0\ 71$	8 61 30 65 38 67 48 70 53 72 58 $x=\frac{44}{28}=1.57$	60 78 72 77 78 75 80 75 78 72 78 $x=\frac{27}{45}=0.6$	70 68 71 68 71 68 72 68 70 68 68 $x=\frac{28}{40}=0.7$	20 50 40 52 45 52 48 50 48 50 50 $x=\frac{20}{30}=0.67$	37 75 65 80 70 77 73 73 72 73 75 $x=\frac{26}{47}=0.55$	65 77 73 77 76 75 78 75 77 75 77 $x=\frac{35}{40}=0.9$	28 83 63 80 70 78 83 75 87 73 87 $x=\frac{41}{32}=1.28$	45 88 62 90 75 90 85 90 88 90 88 $x=\frac{48}{42}=1.14$	42 90 75 90 90 88 90 88 90 88 90 $x=\frac{48}{40}=1.2$	0 5 + 5 + 5 + 5 + 5 5 $x=\frac{0}{5}=0$

TABLE 3.—*Continued*

Comparison of Results in Gas-Production from Sugars by Bacillus Anthracis Symptomatici and Allied Organisms *

Inulin	Glycogen	Starch	Glycerin	Adonite	Sorbite	Dulcite	Mannite	Salicin	Amygdalin	Phlorizin	Plain Carbohydrate-free Broth
B. oedematis-maligni, I											
0 6 0 7 + 7 + 7 + 7 6 $x=\frac{1}{6}=0.17$	0 7 + 7 + 7 + 7 + 7 + $x=\frac{0}{7}=0$	0 8 + 8 5 8 5 8 8 8 8 $x=\frac{0}{8}=0$	0 32 + 34 20 34 25 37 28 37 30 $x=\frac{11}{26}=0.42$	0 5 0 5 + 5 + 5 + 5 5 $x=\frac{0}{5}=0$	0 14 20 + 17 6 17 10 17 12 19 14 20 $x=\frac{8}{12}=0.67$	0 5 0 5 + 5 + 5 + 5 5 $x=\frac{0}{5}=0$	0 7 0 9 + 9 5 9 7 9 7 $x=\frac{0}{9}=0$	0 5 8 0 5 0 5 + 7 + 8 5 8 $x=\frac{0}{[illegible]}=0$	0 5 0 7 0 7 + 7 + 7 + $x=\frac{0}{7}=0$	0 0 0 0 0 + 0 + 0 + 0 $x=\frac{0}{+}=0$	0 8 0 8 + 8 + 8 6 8 6 $x=\frac{0}{8}=0$
B. oedematis-maligni, II, (Novy 1-ME II)											
0 7 0 7 + 7 + 7 + 7 7 $x=\frac{0}{7}=0$	0 + 0 + 0 + + + + + + $x=\frac{0}{+}=0$	0 7 5 7 5 7 7 7 7 7 7 $x=\frac{0}{7}=0$	0 30 0 35 + 40 13 43 19 43 24 $x=\frac{16}{27}=0.6$	0 7 0 7 0 7 + 7 + 7 7 $x=\frac{0}{7}=0$	0 12 15 + 13 8 13 9 13 10 14 12 15 $x=\frac{5}{10}=0.5$	0 8 0 8 + 9 5 9 8 9 8 $x=\frac{0}{[illegible]}=0$	0 10 0 10 + 10 7 10 8 10 8 $x=\frac{1}{9}=0.11$	0 5 10 0 8 0 8 + 8 + 9 5 10 $x=\frac{0}{10}=0$	0 9 0 10 0 10 + 10 8 10 9 $x=\frac{0}{10}=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 6 0 7 + 7 + 7 + 7 6 $x=\frac{0}{7}=0$
B. botulinus											
0 + 0 + + + + + + + + $x=\frac{0}{+}=0$	0 + + + + + + + + + + $x=\frac{0}{+}=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 22 0 23 10 25 15 28 17 28 20 $x=\frac{10}{18}=0.55$	0 + 0 6 0 6 + 6 + 6 + $x=\frac{0}{6}=0$	0 13 23 + 15 8 17 10 19 11 21 13 23 $x=\frac{11}{12}=0.92$	0 5 0 6 + 6 + 6 5 6 5 $x=\frac{0}{6}=0$	0 10 0 10 + 10 8 10 9 10 9 $x=\frac{1}{9}=0.11$	0 5 8 0 7 0 7 + 7 + 8 + 8 $x=\frac{0}{8}=0$	0 8 0 9 0 9 + 9 5 9 7 $x=\frac{0}{[illegible]}=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 10 0 10 + 10 5 10 8 10 8 $x=\frac{0}{10}=0$
B. coli (control)											
0 + 0 + + + + + + + + $x=\frac{0}{+}=0$	0 5 + 5 + 5 + 5 + 5 + $x=\frac{0}{5}=0$	0 18 0 38 0 47 0 50 + 50 7 $x=\frac{11}{39}=0.26$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	64 83 90 84 90 81 87 77 82 77 83 $x=\frac{20}{57}=0.35$	0 95 65 95 90 95 92 90 92 90 95 $x=\frac{25}{65}=0.38$	50 73 75 75 76 73 75 73 71 73 73 $x=\frac{23}{50}=0.46$	0 51 0 52 35 52 48 50 48 50 51 $x=\frac{15}{35}=0.42$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 0 0 0 0 0 0 0 0 0 0 $x=0$	0 7 0 7 + 7 5 7 7 7 7 $x=\frac{0}{7}=0$

TABLE 4

Summarized Readings Taken From Table 3

		Dextrose		Levulose		Mannose		Maltose		Saccharose	
		CO₂	H	CO₂	H	CO₂	H	CO₂	H	CO₂	H
B anthracis-symptomatici..	2	15	15	49	20	4	10	36	12	0	5
	3	40	12	60	12	8	14	37	13	2	9
	4	40	20	36	14	4	5	43	22	2	10
	5	23	10	21	13	2	8	28	18	3	12
	6	42	13	54	22	3	10	44	17	3	7
	10	24	9	50	13	2	7	55	22	3	12
	12	5	5	8	6	3	9	33	18	0	11
	48	32	8	20	8	3	9	41	13	1	7
	60	43	10	58	12	1	6	57	18	2	9
Total		264	102	346	120	30	78	374	153	16	84
Average		29.3	11.3	38.4	13.3	3.3	8.4	41.6	17	1.8	9.3
Average total gas		40.6		51.7		11.7		58.6		11.1	
CO₂/H		2.59		2.9		0.39		2.56		0.19	
Ghon-Sachs bacillus		8	16	3	7	0	0	6	18	0	8
Total gas		24		10		0		24		8	
CO₂/H		0.5		0.42		0		0.33		0	
B. oedematis-maligni	421	21	10	10	5	4	9	13	52	4	11
	485	14	10	11	9	2	10	33	17	3	10
	558	12	8	9	5	3	12	51	18	2	11
	560	5	3	15	5	4	10	14	5	10	18
Total		52	31	45	24	14	41	111	92	19	50
Average		13	7.8	11.3	6	3.3	10.3	27.8	23	4.8	12.5
Average total gas		20.8		17.3		13.6		50.8		17.3	
CO₂/H		1.68		1.88		0.32		1.2		0.38	
B. oedematis-maligni II		12	4	9	7	1	3	21	6	1	4
Total gas		16		16		4		27		5	
CO₂/H		3.0		1.3		0.33		3.5		0.25	
B. botulinus		9	5	10	5	0	6	11	8	2	8
Total gas		14		15		6		19		10	
CO₂/H		1.8		2.0		0		1.38		0.25	
B. coli		30	33	30	42	28	40	41	32	48	42
Total gas		63		72		68		73		90	
CO₂/H		0.97		0.71		0.70		1.28		1.14	

dioxid present, but the gas formed was explosive when mixed with air. In the first trial, conducted like the second except for the difference in seed cultures, the same strain of B. coli and several other strains produced no gas from the broth during 11 days' incubation. In the second trial, for which the seed used had been grown and trained (with some difficulty) in the sugar-free broth, colon bacilli, as well as the majority of the spore-bearing anaerobes, produced gas from the broth, as is shown in the tables. This suggests the influence that training of the stock cultures may have on the gas-producing characteristic.

Our results thus far show that the minor sugars, etc., are of little value in the differentiation of the strains on the basis of total production of gas. Strains of the same organism obtained from various sources differ from one another considerably in their ability to attack the several carbohydrates and form gas

TABLE 4.—*Continued*

SUMMARIZED READINGS TAKEN FROM TABLE 3

Glycerin		Sorbite		Salicin		General Average			
CO_2	H	CO_2	H	CO_2	H	Av. Total	CO_2	H	CO_2/H
0	10	35	21	3	10	30.7	17.8	12.9	1.38
0	8	4	11	2	10	29.0	17.9	11.1	1.60
0	11	4	14	4	18	30.9	16.6	14.3	1.17
0	13	4	10	0	13	22.2	10.1	12.1	0.84
0	9	13	20	0	5	32.8	19.9	12.9	1.54
0	11	14	21	19	23	34.4	18.4	16.0	1.15
0	9	9	22	4	23	20.7	7.8	12.9	0.60
0	8	47	21	5	20	30.4	18.6	11.8	1.57
0	10	8	18	0	5	32.1	21.1	11.0	1.92
0	89	138	158	37	127				
0	9.9	15.4	17.6	4.1	14.1	29.4	16.7	12.7	1.32
9.9		33		18.2					
0.0		0.9		0.28					
0	+	0	0	0	0	8.6	2.1	6.5	0.33
+		0		0					
0		0		0					
0	8	6	18	0	19	23.8	7.3	16.5	0.44
11	16	32	28	3	28	29.6	13.6	16.0	0.85
0	9	8	18	8	25	24.9	11.6	13.3	0.88
11	26	8	12	0	8	19.3	8.4	10.9	0.77
22	59	54	76	11	80				
5.5	14.8	13.5	19	2.8	20	24.4	10.2	14.2	0.72
20.3		32.5		22.8					
0.37		0.71		0.14					
16	27	5	10	0	10	17.0	8.1	8.9	0.92
43		15		10					
0.60		0.50		0					
10	18	11	12	0	8	15.6	6.6	9.0	0.76
28		23		8					
0.55		0.92		0					
0	0	20	57	15	35	61.6	26.5	35.1	0.76
0		77		50					
0		0.35		0.42					

from them. Again, some strains produce a certain amount of carbon dioxid while others produce none. Both these factors vary somewhat from experiment to experiment in the carbohydrate media with which only small amounts of gas are produced.

A study of the coefficients obtained with the carbohydrates from which the larger amounts of gas are formed—that is, dextrose, levulose, mannose, maltose, saccharose, glycerin, sorbite, and salicin—shows several interesting results. In general it may be said that the malignant-edema strains produce less total gas, with a smaller proportion of carbon dioxid, than is produced by the symptomatic-anthrax strains. The Ghon-Sachs bacillus produces only a small amount of carbon dioxid—with most sugars, none at all.

With dextrose, levulose, and maltose, symptomatic-anthrax strains produce much the greater amount of gas, a major part of which is carbon dioxid.

With glycerin, symptomatic-anthrax strains produce no carbon dioxid at all, while malignant edema strains do. The latter also produce twice as much gas in this medium.

Malignant-edema-II strains, though producing a smaller amount of gas, produce a greater proportion of carbon dioxid than Malignant-edema-I strains.

The gas shrinkage, especially in the gas of B. coli, as suggested by Keyes,[5] would seem to be largely due to the diffusion of the more soluble carbon dioxid through the liquid to the open arm where the concentration is nearly zero. He finds that cultures of B. coli in a vacuum apparatus show a larger amount of gas and a larger proportion of carbon dioxid than do cultures of the same organism in ordinary fermentation tubes. Since the action of B. coli is over in a comparatively short time and the collected gas stands for several days, there must be considerable loss by diffusion.

None of the 9 strains of the bacillus of symptomatic anthrax produced more hydrogen than carbon dioxid with dextrose. With this sugar, an average coefficient CO_2/H of 2.59 was obtained, a result contradicting the statement of Franklin and Haslam,[6] that

TABLE 5

RESULTS OF TESTS FOR SUBSTANCES GIVING RISE TO CHARACTERISTIC PUTRID ODORS IN CULTURES

Strain	No.	Hydrogen Sulfid	Methyl Mercaptan	Indol
B. anthracis-symptomatici	2	+	+	+++
	3	+	+	++
	4	+	+	++
	5	+	+	++
	6	+	—	++
	10	+	—	+
	12	+	+ (faint)	+
	48	+	+ (faint)	+
	60	+	+	+
Ghon-Sachs bacillus	1	—	—	±
B. oedematis-maligni	421	+	+	±
	485	+	+	++
	558	+	+	++
	560	+	+	++
B. oedematis-maligni, II		+	+	+
B. botulinus		+	..	+
B. coli		—	±	+++
B. proteus		+	+ (faint)	+
Blank control		—	—	—

Tests used: for hydrogen sulfid, the lead acetate test; for methyl mercaptan, the isatin test[7]; for indol, Salkowski's reaction.

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[5] Jour. Med. Research, 1909, 16, p. 73.

[6] Franklin and Haslam, Jour. Infect. Dis., 1916, 19, p. 462.

[7] Bauer, Ztschr. f. physiol. Chem., 1902, 35, p. 346.

blackleg always produces more hydrogen than carbon dioxid with this sugar. It is not stated in their communication at what stage in the fermentation the gas was analyzed; a premature reading, such as one 72 hours after inoculation, would give a different carbon-dioxid factor from that obtained at the end of gas-production. Also the arbitrary use of a 4% sugar medium may have had some influence on the factors obtained.

Emphasis has frequently been placed on the characteristic odor of symptomatic-anthrax cultures as a means of differentiating them from those of malignant edema.[3, 4] The general opinion is that cultures of symptomatic anthrax are of an aromatic acid odor, particularly like that of butyric acid, while those of malignant edema are of a vile putrid odor. The results of the olfactory test on our cultures agree with this general opinion when applied to young cultures, but with old cultures the reverse obtains. However, tests made for the substances credited with the production of the vile odor—indol, hydrogen sulfid, and methyl mercaptan—show that strain members of both the symptomatic-anthrax and malignant-edema groups originate these substances.

SUMMARY.

B. anthracis-symptomatici produces more gas from dextrose, levulose, and maltose than B. oedematis-maligni, and a greater proportion of this is carbon dioxid. This is illustrated in the following summarized table (averages for 9 strains of B. anthracis-symptomatici, and for 4 strains of B. oedematis-maligni).

	Dextrose	Levulose	Maltose	Average
		B. anthracis-symptomatici		
CO_2	29.30	38.4	41.60	36.40
H	11.30	13.3	17.00	13.90
Total gas	40.60	51.7	58.60	50.30
CO_2/H	2.59	2.9	2.56	2.65
		B. oedematis-maligni		
CO_2	13.00	11.30	27.8	17.30
H	7.80	6.00	23.0	12.80
Total gas	20.80	17.30	50.8	29.60
CO_2/H	1.68	1.88	1.2	1.41

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[3] von Hibler, Handb. d. pathogen. Mikro organismen, 1912, 4, p. 808. [4] Hiss and Zinsser. Bacteriology, p. 470.

Training of seed cultures influences the gas-production of these organisms considerably.

Substances credited with the putrid odor are produced in cultures by strain members of both groups.

The gas-production of most of the strains occurs late and progressively up to about the 10th day. In some media, gas-production continues to a slight degree for some time after this.

An explosive mixture of gases, apparently containing hydrogen and no carbon dioxid, may be produced from "carbohydrate-free" broth.

101480

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APPARATUS FOR USE IN EXAMINING FECES FOR EVIDENCES OF PARASITISM.*

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In a previous paper,[1] later reissued with addenda,[2] the writer has given a comparative study of methods of examining feces for evidences of parasitism. In that study, the various methods which had been advocated were described and it was pointed out that these numerous modifications of technic had as their object the concentration of parasite eggs and the facilitation of the detection of the eggs or of other parasitic material, such as segments, entire worms, etc. Such concentration is obtained by taking advantage of the differences in physical, chemical, and biological properties between parasitic and non-parasitic material. These are mostly differences in specific gravity, size, physical, and chemical solubility, adhesiveness, and capacity for growth and development.

In comparative tests, the writer found the most satisfactory procedure to lie in the middle course between the old and crude smear method and the rather elaborate refinements which call in the use of various chemicals. Very dependable results were secured within a quite reasonable time by taking advantage of the purely physical differences existing between parasitic and non-parasitic material. These results were obtained by the use of screens to remove coarse material and use of a liberal amount of water to remove soluble coloring matters and fine matter in suspension. The use of some apparatus for breaking up fecal masses was advocated, and also the use of a centrifuge to save the time required for sedimentation of samples.

In the years since these studies were published, the writer has continued to find such a method as that outlined above quite satisfactory, but has found certain changes in the apparatus advantageous. The method I am using at present is as follows:

Rather large fecal samples, 2 or 3 ounces, are used wherever

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[1] Bu. Anim. Indust. Bull. 135, 1911, 36 pp.

[2] Idem, with addenda, 1912, 42 pp.

possible, instead of the classical sample, "about the size of a walnut." The reason for this is that in a general way it is desirable in our work to ascertain whether any parasites are present, not merely whether there is a massive infestation. By using large samples and concentrating the eggs present, it is possible to detect very light infestations. The use of the much simpler smear method, taking a small amount of fecal matter on the end of a toothpick and smearing it on a slide with a drop of water, will perhaps serve the purposes of the busy practitioner who cannot take the time and trouble to use more accurate methods, but it should be understood that the chance of detecting light infestations in this way is much less than where the more elaborate methods are used. Where a physician is primarily concerned with detection of massive infestation with hookworm, the smear method will probably serve very well, especially since there will be clinical indications of such infestations in nearly all cases. But even here it should be noted that in communities where there are massive infestations with hookworm, there will usually be simple or combined infestations with ascarids, whipworms, *Strongyloides* or tapeworm also present, and it is desirable to detect these even in light infestations. Ascarids are regarded as rather innocuous by some physicians, but the writer would prefer an infestation with a moderate amount of hookworms to an infestation with ascarids in any amount from one up. The large size and wandering habits of these worms make them a potential source of serious danger. Cases have been reported where these worms have entered the ducts of the liver and pancreas, crawled up the esophagus and down the trachea to the lungs, or through the eustachian tube and out of the ear; where a single worm has caused enterospasm necessitating operative relief; where they have perforated the intestine and caused peritonitis; and even the not uncommon occurrence of vomiting one or more of these worms, which are about the size of a lead pencil, is a prospect one might well face with hesitancy. In view of these facts it would seem as well worth while to employ means to ascertain with the greatest possible accuracy whether worms were present, as to employ the utmost accuracy to ascertain whether *Plasmodium* or *Treponema* or *gonococcus* or *B. tuberculosis* were present. In any case accurate information is the thing sought.

Large fecal samples are also desirable for the reason that tapeworm infestation is not uncommonly present without eggs being present in the feces. It occasionally happens that segments of tapeworms are present and can be detected by using large samples and screening, and that the same fecal sample will show no tapeworm eggs owing to the fact that the segments have not ruptured and released the eggs. This is less apt to be the case for infestation with *Tænia*, owing to the position of the uterus in that species, though it is a possibility there also, but is not uncommonly the case for such cestodes as *Dipylidium*, where the eggs are retained in egg capsules in the parenchyma of the segment. In addition to tapeworm segments, the screens will occasionally retain entire nematodes or cestodes which are being passed, and of course the same would be true for flukes.

In the method previously given for fecal examinations, the writer described the use of a "milk shaker," of the type where the glasses are shaken up and down, for use in breaking up fecal samples. This apparatus, motor-driven, was very satisfactory in most respects, but had the disadvantage of being very noisy. A substitute for this machine was found in the newer type of mixer, in which a small motor drives a vertical shaft terminating at the bottom in a smooth-edged hexagonal disk. In this machine, which is shown at the right of Fig. 1, the motor head is lowered so that the disk is near the bottom of the inside of the bottle containing the feces, the current automatically turning on and spinning the shaft and disk as the motor head is lowered. This machine seems to be an improvement over the old type for breaking up feces. It works more rapidly and is practically noiseless. It does not damage nematodes or isolated tapeworm segments which may be present, though it does sometimes break tapeworm strobilæ which may be in the feces. For the purpose of breaking feces to release contained worm eggs, it gives entire satisfaction. The old shaking machine worked with the bottle corked and was odorless in operation. The new machine works with the bottle uncorked, and does release unpleasant gases, but we have carried a rubber tube from the suction pipe in the laboratory and terminated the tube in a small funnel, shown in the illustration, which is held by rubber bands near and above the mouth of the bottle, and find that the suction serves to carry away practically all of the odors. The bottles used have a capacity of 400 mils, and water is added to the feces to make the total content about 300 mils.

The feces having been thoroughly disintegrated with the mixer, they are next poured through a set of sieves. In my former papers I described a sieve made by soldering brass screening to round tin pans from which the tin bottoms had been cut out except for a flange to which the screen was soldered. In the addenda, I noted that Dr. Cobb, of the United States Department of Agriculture, had devised some oblong screens which he had found satisfactory. In making up screens for work here, the round form was abandoned and a screen which is approximately square was made up in the shops. These screens, shown in Fig. 1, are of copper, with inside dimensions about 7⅞ inches (20 cm.) square. They are made of two copper strips, swaged together at two diagonal corners, the top half inch of the metal being bent over and doubled back against the side on two opposite sides to form a reinforced rim, and being bent out and back on the other two sides to form a reinforced flange which carries the sieve in a rack. This flange is ¼ inch wide. The copper sides are two inches high. On the bottom of the sieves the metal is bent in to form a flange ⅜ inch wide for the attachment of the brass screening, which is soldered to this flange. The screens used have mesh apertures of 6, 8, 10, 14, 16, 20, 60, 100 and 120 to the inch, the latter being about the finest screen that will permit of the passage of the eggs of practically any worm parasite. Each sieve has a number stamped in the front side showing the number of mesh apertures to the inch in its screening. A solid-bottomed copper pan, of the same shape and dimensions as the screens, completes this part of the equipment.

In previous publications, the writer has advocated the use of a set of screens which could be "nested," and which were used in this fashion; the feces were poured through the screens when the latter were set one within another in proper order in a glass dish. While still in the service of the United States Bureau of Animal Industry, I abandoned this plan in favor of the use of a rack, similar to the right-hand one of the two racks shown in Fig. 1, constructed with two solid board sides and with the boards grooved so that the edges of the circular pans could be slipped into the grooves. The same type of rack serves even better with the present rectangular sieves, and has been improved in the left-hand rack shown in Fig. 1, which is a skeleton construction, lighter and less inclined to warp. This latter rack is made of four upright wooden pieces joined by a series of transverse pieces on the sides and back, and by a top and bottom piece in front. In examining feces for eggs, this large rack, with a carrying capacity of nine sieves and one solid-bottomed pan, is used, the coarsest screen being at the top and the pan at the bottom. When examining feces for worms passed after the administration of anthelmintics, the smaller rack, with an equipment of six of the coarser screens, using the 60 mesh for the bottom screen to guard against the escape of any worms, is used. The racks serve as storage containers for the sieves and pan when these are not in use.

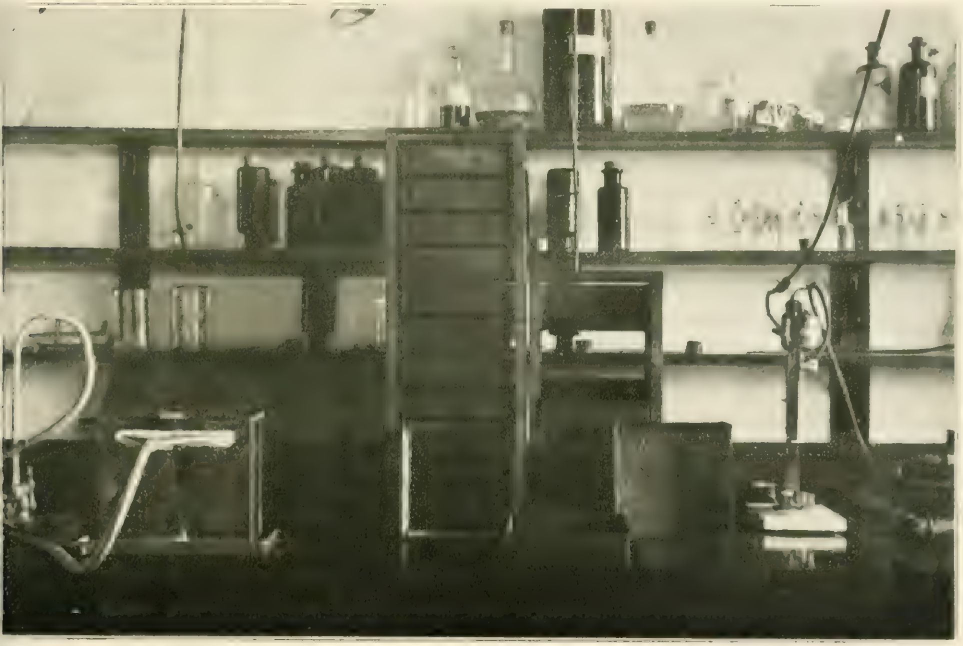

Fig. 1.—Apparatus for comminuting and screening feces, and for washing screens.

When examining feces for evidences of parasitism in the shape of eggs or larger material, the feces broken up by the mixer are poured through the set of screens in the large rack and the screened feces collected in the pan. The screens are washed by running clear water through them until the pan is perhaps half full. This pan has a capacity of two liters. Then the pan is pulled out and the contents decanted, sedimented, washed and centrifuged to suit the convenience and desires of the operator. The end result is a slide on which there is a minimum of foreign matter, and this foreign matter and the eggs alike are washed clean and stand out for just what they are in the clean water from which soluble matter and fine suspended matter have been removed. Such a slide can be examined with a maximum certainty of detecting eggs present and with a minimum amount of eye strain.

After the pan is taken from the rack, the remaining sieves are washed by running water through from the top until the water from the last sieve comes away clean; in other words until all the soluble coloring matter and matter fine enough to pass the screens are removed. Then the sieves are examined one at a time in a glass tray of clean water. Parasitic and non-parasitic matter are now washed clean and appear in their true shapes and colors. There is little likelihood of mistaking vegetable or animal material for worms, and little likelihood of overlooking worms. Substantially the same thing is accomplished in examining feces with the smaller rack and fewer screens to detect worms passed after anthelmintics.

It is necessary that a screen equipment of this sort be kept clean and free from contamination that would give misleading results. To cut down the work of cleaning these screens, the apparatus shown at the left of Fig. 1 was constructed. This is essentially a copper box, 3½ inches wide, 9 inches high, and 10 inches long; just large enough to wash one screen at a time. Water is sprayed in through the fan-shaped nozzle at the back, the aperture being cut down by solder plugs at intervals to increase the force of the water. On the front of this box are three overflow lips at the top and a small tube at the bottom; light material floats off through the upper lips, and heavy material, such as sand, is washed out at the bottom. The box is provided with a lid to prevent splashing and is set in a tray to drain any overflow to the sink. In practice, sieves are first rinsed under the overflow and then put in the box. One sieve is washed while the next is being examined. By using very hot water, the sieves are not only cleansed, but practically sterilized. The mixer is very easily cleaned by holding a damp cloth against the shaft or disk while these are spinning.

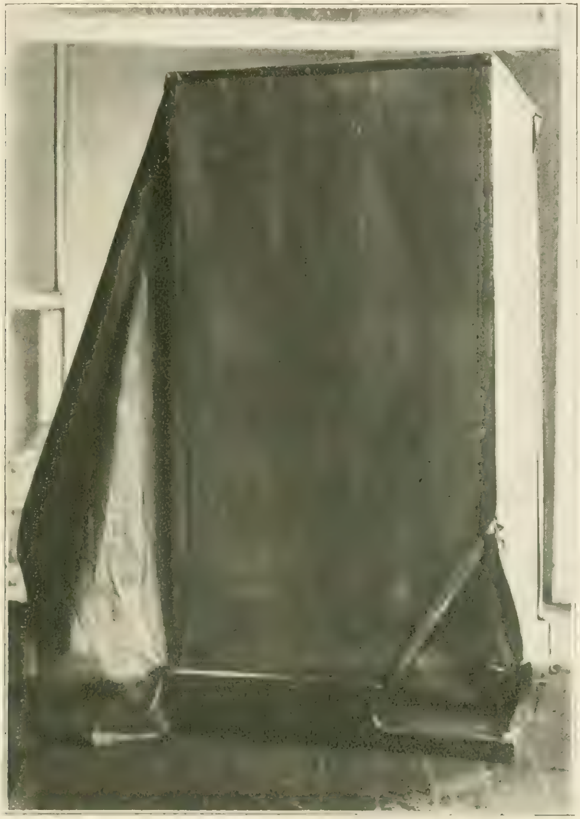

Fig. 2. -Exterior view of dark cell for microscopic work.

So far as sterilization is concerned, however, it is advisable to have formaldehyde, a coal-tar disinfectant, or some other adequate disinfectant, added to the original fecal sample whenever feasible. This is valuable for sterilization and also aids in abating the odor. Where plenty of water is used, the odor is a matter of small moment, as the substances responsible for it are soon washed away.

The writer has long been in sympathy with Dr. Cobb's idea that in doing microscopic work it was advisable to illuminate only the object under the microscope and to cut out, as far as possible, the illumination of the microscope and other extraneous objects in the room. However, the equipment which Dr. Cobb has used to attain his object is rather expensive, the cost of equipping each window with the very satisfactory apparatus which he has devised being about $80.00 to $100.00 two years ago, and probably more now. A similar result may be attained by the use of the simple

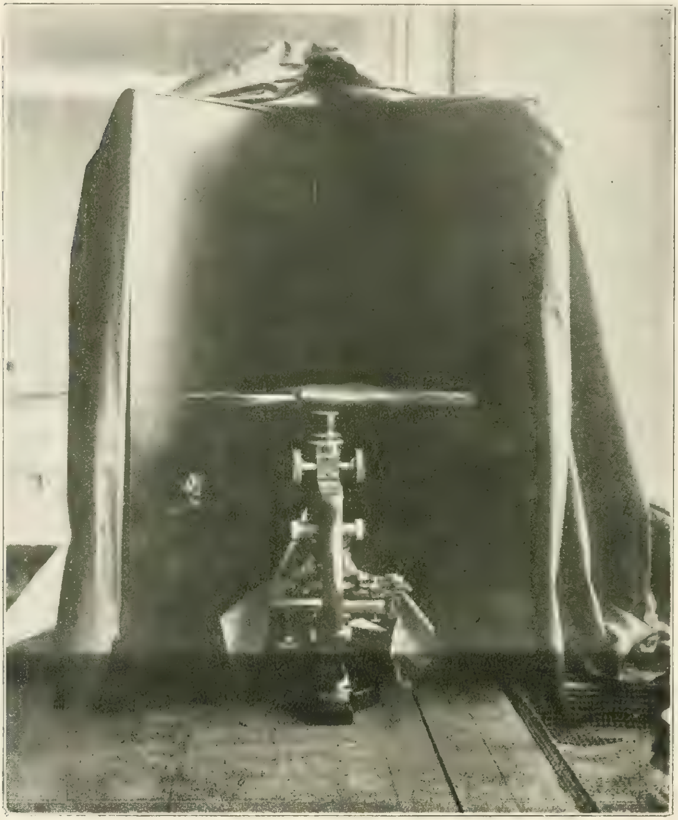

Fig. 3.—Interior view of dark cell for microscopic work.

and inexpensive device which is shown in Figs. 2 and 3. This device consists of two boards, each 2 feet long and 1 foot wide, joined at a right angle along the long edges by screws. As shown in Fig. 2, the boards are sawed out at the bottom of this right-angled union to a height of 8 inches, and a reinforcing board fastened along the bottom of the large boards and across the bottom of this aperture to prevent the structure from falling. To further prevent falling, the angle formed by these reinforcing boards is held under a bent nail. A strip of black cloth (lining) is nailed to the edges of the aperture and carried back to the stage of the microscope, permitting light to fall on the mirror. A large piece of black cloth (lining) is nailed to the free edges and top of the large boards, and when this is dropped over the microscopist, it forms a dark chamber which is very restful to the eyes in examining fecal samples for parasite eggs. Midway between the top and bottom of the boards is a triangular shelf, about a foot long on its free edge, and with the free edge hollowed out, beveled and padded to make a head rest. The woodwork of the entire interior of the dark cell is painted black. With this very simple and cheap device one can examine fecal samples for hours and feel very little of the neck and eye-strain that would result from the same amount of work in the open and with the head unsupported. There is only a certain amount of nervous energy available for work; and if this energy is partly dissipated in unprofitable strain on the neck and eyes and in the distraction incidental to seeing the objects and action in the room, there is that much less available for concentration on the work in hand. The use of this apparatus gives a distinct sense of concentration and a feeling of greater satisfaction in what otherwise quickly becomes rather tedious work. As Dr. Cobb has pointed out in his papers, few microscopists seem to appreciate the value of providing suitable and comfortable conditions under which to work, but this does not detract from that value.

When the cloth at the aperture for illumination of the microscope mirror is pushed down and the cloth at the top and back of the dark chamber dropped, the microscope is covered and protected. This arrangement gives adequate protection to the instrument and is handier than the use of a microscope case, as the microscope is always in place ready for use.

ADDENDUM.

Since the above paper was published in the *Journal of Laboratory and Clinical Medicine*, the writer has modified the apparatus slightly to facilitate the removal of malodorous gases when using the mixer. Instead of using a funnel loosely attached to the mixer to collect the gases, a copper cap is used. This cap has a neck which is attached by set-screws to the stationary head just above the rotating shaft and below the motor, the attachment being made air-tight by a small rubber gasket; from this neck the cap flares out like a funnel to a position just beyond the edge of the neck of the bottles, and there drops a rim extending down a half-inch or so below the bottle neck; a small copper tube is soldered into the flare of the cap, and the rubber tube attached to the suction pipe is connected to this. The suction draws the air up between the bottle neck and the enclosing rim, and this air and the released gases from the bottle are carried off through the suction pipe.

101487

Studies from the Research Laboratory
Parke, Davis & Co.
Reprint No. 120, 1917

THE LONGEVITY OF ADULT ASCARIDS OUTSIDE THE BODY OF THE HOST.

ITS BEARING ON ANTHELMINTIC TREATMENT.

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Wharton[1] has recorded experiments in which he kept the eggs of the common roundworm of man, *Ascaris lumbricoides,* alive in Kronecker's salt solution (physiologic sodium chlorid solution to which 0.06 gm. of sodium hydroxid per liter is added) for from six to twelve days. While I was in the service of the federal Bureau of Animal Industry, I made some tests to determine the length of time that the common roundworm of the pig, *Ascaris suum,* could survive outside the body of the host. A number of these worms had been collected and thrown into about a 5 per cent. dilution of liquor formaldehydi and left for a few minutes. They were taken out of the formaldehyd and nine of them placed in Kronecker's solution and nine placed in physiologic sodium chlorid solution. The height at which the solution stood when made up was marked on the glass dishes containing them, and once a day distilled water was added to replace that lost by evaporation. The condition of the worms was noted every day except Sunday, and the room temperature noted every day except Sunday from the fifth day to the end of the experiment.

During the course of the experiment, the room temperature ranged from 27.5 to 30 C. (from 81.5 to 86 F.). The last survivor in the physiologic sodium chlorid solution, a male, was alive on the fourteenth day after removal from the host, and was found dead on the fifteenth day. Three females survived for nineteen days in Kronecker's solution; two of these survived for twenty-four days, and one of these was alive on the twenty-sixth day after removal from the host and was found dead on the following day. In another experiment in which ten specimens of *Ascaris suum* were kept in physiologic sodium chlorid solution, one worm was still alive on the fifteenth day. It appears, then, that ascarids may survive for fifteen days in physiologic sodium chlorid and for twenty-six days in Kronecker's solution.

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[1] Wharton: Philippine Jour. Sc., 1915, **10,** 19.

The particular interest which attaches to this experiment is its bearing on a very common idea in connection with anthelmintic treatment. It is quite commonly believed, and the belief is shared by many physicians and veterinarians, and is expounded in reputable medical literature, especially the older literature, as well as in the advertisements of commercial houses, that when a patient infested with worms is fasted for twelve or twenty-four hours, the worms become hungry as a result of this lack of food, and that when an anthelmintic is administered, especially when it is given in some such vehicle as milk, the hungry worms will ingest the anthelmintic and thereby become poisoned.

There are two or three objections to such a belief. In the first place, when these worms can survive for twenty-six days in such an unnutritious medium as Kronecker's solution, it is unlikely that they would suffer the pangs of hunger after twelve or twenty-four hours in contact with the collapsed walls of the intestine and the relatively abundant content of food, epithelial débris and secretions which is still present after such an interval. Moreover, it is known that the digested or partly digested food in the lumen of the host intestine is not the only food of parasitic worms, and it may constitute in some cases a very small part or no part at all. Garin says that nematodes of the digestive tract live at the expense of the wall of the digestive tract in all cases, and not on food in the lumen. According to Garin, some worms (*Heterakis papillosa*) live on mucus and intestinal juices; others (*Oxyuris, Ascaris lumbricoides, Ascaris suum*) live on epithelial cells; others (*Ascaris rotundata, Physaloptera clausa, Habronema microstoma*) live on lymphatic cells and lymph; others (*Strongylus, Ancylostoma, Graphidium, Trichuris*) live on blood. He states that the attack on the mucosa may be mechanical (with teeth, etc., as in the case of hookworm) or chemical (by digestive secretions, as in the case of whipworms). Much remains to be learned in this connection, but it is known that such forms should suffer no inconvenience whatever during the period in which the host animal is deprived of food. Finally, it is not necessary to suppose that anthelmintics must be voluntarily ingested by the parasitic worms in order that the worms may be destroyed. It is likely that they are either ingested without volition or that they exercise their lethal effects in some cases by absorption, as may be the case with such volatile anthelmintics as chloroform.

In any case, the preliminary fasting, as well as the preliminary purgation, previous to anthelmintic treatment is, of course, a very desirable procedure. But its utility lies in the fact that it removes the bulky food mass which might otherwise protect the worm against the anthelmintic action.

CORRECTION.

In the first sentence of this paper as published and as reprinted, it is stated that Wharton kept the *eggs* of the roundworm, *Ascaris lumbricoides,* alive in Kronecker's solution for six to twelve days. As the rest of this paper suggests, Wharton kept the *adult worms* alive for six to twelve days.

101486

Studies from the Research Laboratory
Parke, Davis & Co.
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FACTS AND FALLACIES IN DISINFECTION.*

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One of the most important problems confronting the well informed health officer is that of cleaning up or preventing infection. Whatever measures he takes, these should be supplemented by an intelligent use of some chemical disinfectant. What shall he use? A new substance is proposed and gains a certain degree of popularity only to be relegated to a secondary place in favor of one still newer. One is likely to lose sight of the virtues of the older disinfectants in this maze of new products.

It is advisable to glance over the field occasionally to discover the advantages and disadvantages of these various substances; for while few are valueless only the best for the specific purpose is to be recommended.

A disinfectant is often overrated because in the hands of a careful user exceptionally good results were obtained. Some are discredited because of being used under improper or inappropriate conditions. Almost any substance may, under certain conditions, appear to be a disinfectant when in reality the conditions and not the substance were unfavorable to the development of the organisms.

If bacteria are transplanted from contact with a substance which reduced their vitality, to a culture medium slightly unfavorable for growth, an erroneous impression will often be obtained as to the germicidal value of the substance in question.

This actually occurred in testing a well known protein compound of silver which in a medium regularly adjusted by addition of hydrochloric acid to an acidity of 1.5, gave a phenol coefficient of 5, while in an unadjusted medium with acidity of 0.8 the coefficient fell to about .5 to 1. (See Protocol 1, Appendix.)

Another occasion for the discredit of a good disinfectant is the erroneous idea in the lay mind that a disinfectant is an insecticide. Formaldehyde fails as a fumigant for destroying insect life, a fact which may partly explain the appearance of adverse reports. (See Protocol 2, Appendix.)

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*Read before the Laboratory Section of the American Public Health Association, Cincinnati, Ohio, Oct. 26, 1916.

The more probable reason, however, for the often expressed disparagement of formaldehyde is an improper use largely because of a misconception of its properties. Dry formaldehyde gas while exceedingly irritating to mucous membrane is almost innocuous to bacteria, the germicidal value being brought out only when in aqueous solution, or probably by the hydrate. It readily polymerizes, so that if slowly generated with insufficient moisture present, the active hydrate is not produced in a proportion to be efficient. (See Protocol 3, Appendix.)

Various improper methods have been proposed and applied for generating the gas from its aqueous solution. Potassium permanganate or chromate by combining with a portion of the agent generates sufficient heat to volatilize the remainder with the necessary water, the amount destroyed not exceeding 20 to 30 per cent. of the original content, while the rapidity and completeness of evolution both of formaldehyde gas and water more than offset the necessary loss.

When lime is used as the heating agent only the heat of combination with water can be permitted, since the lime-water slowly but almost completely destroys the aldehyde forming inert decomposition compounds. The same is true of caustic soda, which is the reagent employed in one or more commercially exploited generators. It is also essentially true if calcium hypochlorite is so employed.

A prompt and more or less violent reaction occurs when a 40 per cent. aqueous solution of formaldehyde is mixed with either of the above named reagents, but careful experiments failed to find effective quantities of formaldehyde among the evolved gases.

The method employed to determine the amount evolved was essentially that described by Frankforter (J. A. C. S., Vol. 28, 1906, p. 1234), the gases being absorbed by an excess of distilled water. By this method 30 per cent. of the theoretical quantity of gas was found to be liberated from a solution by means of potassium permanganate, while only 3 per cent. could be found when caustic soda was the reagent used. Lime is more efficient than caustic soda because a higher degree of heat is evolved in the reaction. Chlorinated lime under the same conditions appeared to evolve only chlorine compounds, no formaldehyde gas being detected in the aqueous solution collected.

Any rapid method by which the unchanged formaldehyde gas can be driven off from its aqueous solution is more or less satisfactory as a means of disinfecting a room by fumigation.

Formaldehyde gas, however, is so volatile that it must be almost immediately redissolved in water vapor or it will be dissipated before an effective strength is obtained. Therefore there are three highly essential factors in a successful fumigation with formaldehyde, namely, very rapid evolution of gas, a percentage of moisture in the air approaching saturation, and absence of air currents. It follows that disinfection should not be attempted on a windy day, that the air must be rendered moist by sprinkling the floor with water about fifteen minutes before fumigation, and if possible that a permanganate or chromate salt be used to develop the requisite heat.

Calcium hypochlorite or bleaching powder is one of the very few substances which spontaneously evolves an efficient disinfecting vapor.

The gas first released is chlorine, which has a very caustic action on fabrics, colors and metals, and is therefore objectionable in most cases.

Hydrogen peroxide has had a varied reputation. Reputed very highly by some, it is considered worthless by others. It all depends on the point of view and the way in which it is used.

With a phenol coefficient of .03 it is as strong as a 3 per cent. solution of phenol and therefore theoretically capable of destroying almost all infectious organisms. Practically, however, it is so rapidly destroyed in contact with organic matter that no great dependence can be placed on it as a disinfectant unless used repeatedly in considerable quantities. It has a value, however, all out of proportion to its germicidal action, in that as an active oxydizing reagent the evolved gas acts as a cleansing agent, the foreign matter in wounds being extruded in a very efficient manner. It is therefore active in proportion to its hydrogen dioxide content, but not primarily as to its phenol coefficient.

Much difference of opinion exists regarding the value of soap as a disinfectant. Tested against bacteria by any of the quasi-official methods a pure, neutral soap has little germicidal action in any practicable strength of solution. A 5 per cent. solution of most soaps is efficient in killing all non-spore-bearing bacteria in a few minutes, but this is not a practicable solution to obtain except with soft soap.

Many soaps, however, are not neutral but decidedly alkaline, and to this ingredient undoubtedly some of the relatively high values may be attributed. (See Protocol 4, Appendix.) A neutral soap, or preferably one that is slightly alkaline, is indirectly valuable because of its cleansing properties, particularly in its action to remove the protecting fatty film on the skin and allow penetration of an active agent. Theoretically a true disinfectant associated with soap is an ideal combination for cleansing and disinfecting the hands and the site of an operation. Practically, however, few of the disinfectants with which we are familiar can be advantageously used with soap either because of the physical properties of the resulting mixture or of chemical reactions impairing the quality of one or both of the two agents. Symes investigating this subject (Antiseptic and Disinfectant Properties of Soap, 1909) found that a 1 per cent. solution of soap—as strong as an average solution can be made—kills some bacteria in ten minutes, but most are not killed after hours of exposure, which is also true even of a 5 per cent. solution. He also found that no added disinfectant greatly enhanced the value of soap except biniodide of mercury, which was very effective for disinfecting instruments and the field of an operation. Plain soap is germicidal toward some bacteria, notably *B. typhosus.* My own experiments (See Protocol 5, Appendix) have demonstrated that the different glycerides produce soaps which differ in their germicidal values, but that no soap can be considered an effective disinfectant unless associated with some other more active agent. The substitution of scrubbing with soap, for fumigation in terminal disinfection, is not a safe practice. The cleaning is to be commended but is not a substitute; it is only an aid to disinfection with a fumigant.

Ethyl alcohol is very largely used as a disinfectant for the site of an operation or injection, but by many it is regarded as of little value. It is generally considered that only the medium strengths of alcohol are active.

Experiments recently carried out verify the results obtained by Harrington and Walker (*Boston Medical and Surgical Journal,* Vol. 148, p. 548) (See Protocol 6, Appendix), who found that "unless the bacterial envelope contains a certain amount of moisture it is impervious to strong alcohol; but dried bacteria in contact with dilute alcohol containing from 30 to 60 per cent. of water will absorb the necessary amount of water therefrom very quickly, and then the alcohol itself can reach the cell protoplasm and destroy it."

It is evident, therefore, that sponging the site of an operation with alcohol after scrubbing with soap is a safe procedure; adding acetone to the alcohol is logical as a fat solvent and is quite commonly practiced. A further aid to disinfection is the addition of 1 to 2 per cent. of a high coefficient coal-tar disinfectant to the acetone-alcohol mixture by which one can obtain a sterilizing solution equivalent to 40 per cent. phenol. (*Journal of Surgery, Gynecology and Obstetrics,* Vol. 21, p. 85.) Such a solution is neither poisonous nor irritating except when the hands are encased in rubber gloves after being treated with the mixture.

The high coefficient disinfectants of coal-tar origin have several factors to contend with in finding a satisfactory position among the known germicidal agents. There is doubt as to the high values assigned to them on the basis of a laboratory test, especially since many coal-tar products have been proved to be almost worthless. While it is true that no ideal method of standardization of disinfectants has been evolved many of the variable factors have been discovered and eliminated, so that there is reason to hope that germicidal agents may some day be as accurately graded as other chemical substances. The tests applied are relative, however, and there is little reason to suppose that a substance found by a careful laboratory test to be 2, 5, or 20 times as efficient as another similar substance would not be found in practice to have the same relative value when used under similar conditions. If the standard is of the same character and the conditions are the same the statement could not be questioned. Unfortunately the standard used for comparison is, although technically similar, actually decidedly different in many respects. Pure phenol has been chosen as the standard with which to measure the activity of all classes of disinfectants, and, while it belongs to the same series as the other phenols of coal tar, there are many conditions which change the resistance of the test organism toward everyting but the standard. This is a generally recognized fact and is a serious defect.

On the other hand, the conditions imposed on the laboratory test are sufficiently severe as to eliminate most of these variants.

Another defect in the method of grading disinfectants by their phenol coefficients is the fact that different organisms have a ratio of resistance different from that of *B. typhosus*—the standard test organism. With some organisms the value of disinfectants is higher and with others lower than their present rating. (See Protocol 7, Appendix.) Because of this and other factors involved, the Bureau of Animal Industry has felt compelled to reject all but pharmacopœial germicides for disinfecting premises possibly infected by hog cholera, foot-and-mouth, and other infectious diseases. This has made necessary the use of the relatively expensive cresylic acid, practically all of which is imported, while thousands of gallons of disinfectants from coal-tar distillates of local production are barred from official use. This is a hardship because of the relatively high cost of the imported cresylic acid and is economically unwise because of its relatively low germicidal value. When properly carried out the germicidal test which establishes the phenol coefficient is of more value than a chemical test which compares phenol content only and takes no cognizance of the difference in character of the different classes of phenols. (See Protocol 8, Appendix.)

In connection with the policy of the Department of Agriculture in recommending disinfectants, a point is worthy of notice that lime is recommended to be mixed with the solution of phenol or of cresol compound in the proportion of not to exceed 1½ pounds to 1 gallon of solution. There is unfortunately no express instruction to use fresh water-slaked lime, which would have a degree of germicidal value in itself. The lime compounds of phenol and cresol have about one-half the germicidal values of the uncombined substances, while the lime mixed with cresol compound not only lowers the value of the cresylic acid, but is itself partly neutralized by the soap, forming a sticky, inert lime soap. Such a practice, while intended for the good purpose of showing where the disinfectant has been applied, would appear to do more harm than good unless as noted above the lime used is fresh stone lime. In that case the freshly slaked lime would be of more value than the phenol or cresol compound. (Protocol 9 and 10, Appendix.)

Freshly prepared calcium hydroxide has a coefficient of approximately 20 based on the fresh lime used. (Protocol 11.) It is highly efficient theoretically, but as a practical disinfectant has only a limited application, because of its insolubility, instability, and because of the residue wherever applied.

The effect of lime and magnesia as they often occur in the diluting water for coal-tar disinfectants and dip is of considerable importance in that the emulsifying agent or solvent is in most cases soap. One part of lime combines with 12 parts of soap, forming an insoluble grease no longer capable of emulsifying or dissolving a saponaceous disinfectant. Without this constituent the active agent is no longer in a finely divided state capable of acting uniformly but separates in a layer of oil which often causes considerable damage by reason of its corrosive character in this condition. Such a water should be corrected by the use of washing soda or caustic soda. When properly treated with one or both of these ingredients the water can be safely used as a diluent for any of the coal-tar dips or disinfectants.

The comparative values of an emulsifiable and a soluble disinfectant is a question that seems entirely profitless. It has nevertheless been claimed (Chick & Martin, *Journal of Hygiene*, 1908, Vol. 8, p. 654) that the former is the more efficient since the finely divided particles of the oil were observed to have a Brownian movement, and, in effect, to bombard the bacteria.

Laboratory experiments fail to verify this hypothesis. These were carried out by using cresylic acid and soap, the ratio between the two being varied to obtain in the one case a complete solution, in the other with less soap a hazy emulsion. Careful tests of these two preparations failed to show any material difference. (See Protocol 12, Appendix.)

Another point in connection with coal-tar disinfectants is the effect of exposure to light.

That this effect is to lower the efficiency is the statement made by Prof. Charles E. Higgins of Ottawa (personal communication), who found a marked decrease in germicidal activity in several samples so exposed for three weeks.

To verify this statement a sample was divided, one part remaining in an amber bottle, the other in a small clear glass bottle exposed to the light, partly direct sunlight.

After one month this was found to have lost about 8 per cent. of its activity as compared with the other part. This is not a negligible loss. It is one that should be guarded against by avoiding undue exposure of disinfectants, especially the sample for assay representing a bulk lot. It may well be that this is, in part, the explanation of disagreements in reports from different tests of the same preparation.

The actual loss, however, is insignificant and is offset by the fact that recommended dilutions are usually much lower than the efficiency of the preparation would permit. (See Protocol 13, Appendix.)

One occasionally wonders what combination of circumstances is responsible for the appearance and retention on the market of certain disinfectants for which high values are claimed and which uniformly fail to show these high values by any of the accepted tests. In the case of one product of this character it is claimed to be ten times as efficient as phenol, while careful tests show a value only slightly in excess of that of phenol. The active constituent appears to be B-naphtol dissolved in glycerine. (See Protocol 14, Appendix.)

Another instance of a product less valuable in general than would be justified by the claims is one which has the recommendation of the hygienic laboratory. It is even called the Hygienic Laboratory Pine Oil Disinfectant. It is prepared from a specially distilled pine oil and the impression is given that any oil so prepared is equally valuable. It is described as if it were a new product, while as a matter of fact similar preparations have been on the market for years. Tests are shown (Public Health Report, October 8, 1915) on which a coefficient of 5 is claimed and a dilution of 1 in 500 recommended for general use, while this same dilution acting on a filtered suspension of *B. typhosus* failed to kill in 15 minutes' exposure. (See Protocol 15, Appendix.)

As an evidence of the possible harm done by such a publication a sample of disinfectant prepared in North Carolina from an oil supposed to be identical with the specifications had a coefficient of 0.5. (See Protocol 16, Appendix.) This producer was justified in expecting a coefficient ten times as high as his product actually possessed, and on the basis of the statement below would be justified in marketing his product and quoting as follows:

(PUBLIC HEALTH REPORTS, OCT. 8, 1915.)

"The new preparation is derived from pine oil, a by-product in the manufacture of turpentine. It is easily prepared by mixing certain portions of the oil with rosin and sodium hydroxide solution, the finished product being a reddish-brown liquid, rather thick and oily in appearance, but free from turbidity. With water it makes a perfectly white emulsion, much resembling milk. It has a pleasing odor, no objectionable taste, and attacks neither fabrics nor metals. It possesses over four times the disinfectant properties of carbolic acid and is altogether non-toxic, so that it may safely be used as a throat spray or mouth wash in solutions of the ordinary strength. The cost of the preparation is remarkably low, as it can be manufactured for less than fifty cents a gallon, solely from products which are produced in this country.

"It is believed that this new compound, which is to be known as 'Hygienic Laboratory Pine-Oil Disinfectant,' will become one of the most useful preparations of that character." Fortunately the high cost of the oil has prevented the flooding of the market with this low grade disinfectant.

A brighter picture is presented by Dakin, who brought into use a valuable form of hypochlorite. While the solution Eusol and the powder Eupad suggested by him have no extraordinary values, the toluene derivative, Chloramine T, has properties which commend it to our attention. No extended examination has been made to determine its general applicability, but germicidal assays by the accepted methods show its value to be 50. (See Protocol 17, Appendix.) The presence of organic matter materially lowers this value, but when used as suggested by Dakin, it is probably a safe disinfectant.

On many occasions there is need for a preservative agent to prevent the bacterial decomposition of solutions of organic substances or drug and glandular extracts. While ethyl alcohol is more or less ideal for this purpose, in some cases it is not applicable either because of its action on an active ingredient or because of its effect on the patient.

Certain of the phenols derived from coal tar are made use of, especially in antitoxin. Where those commonly used are not applicable because of the irritation to tissues, other phenols from high coefficient oil can be substituted to advantage. Their low toxicity, freedom from irritation and high antiseptic values all tend to make them very valuable. (Protocol 18.)

Another substance applicable for this purpose is Chloretone—trichlor-tertiary-butyl alcohol. A saturated solution—about 0.8 per cent.—kills *B. typhosus* after two minutes. It is less effective against spores and moulds, but is almost universally applicable against bacterial decomposition. (Protocol 19.)

When the bacteriologists discovered that certain dyes have a specific staining action on bacteria it was the first step toward applying these dyes as germicidal or at least antiseptic agents. There seems to be no relation between color and germicidal activity, but, regardless of color, the acid dyes appear to be inert and the basic, active.

No exhaustive experiments have been made to determine the value of these substances as disinfectants, but experiments carried out by Churchman (*Journal Experimental Medicine,* Vol. 16, p. 221), Russell (same journal, Vol. 20, 1914, p. 545), Hill and Tabor (*Journal Infectious Diseases,* Vol. 15, 1914, p. 566) show a high degree of antiseptic value.

The low toxicity and freedom from irritation of some of the promising dye products lead to some experiments which showed that in vitro methyl violet has a phenol coefficient of 200 against *Bact. diphtheriæ*. Other dyes, gentian violet, malachite green, methylene blue, showed toward *Bact. diphtheriæ* and *B. typhosus* no remarkable value. (Protocol 20.) When the price of dyes resumes a reasonable level it seems very probable that some selected ones may be found well adapted to internal antisepsis and especially in attempting to control diphtheria and typhoid carriers.

In some cases it seems probable that, while certain of these dyes are strongly antiseptic in vitro, they are not exceptional germicides and, therefore, in the living animal are almost valueless. Unless the microörganism is killed favorable conditions for growth are very soon resumed.

This is to a degree applicable to the chemical substance known as Chinosol, which appears on the market also under the name Pix Cresol (*Journal American Medical Association*, 1911). While it actually has exceptionally high antiseptic value—many times more powerful than phenol—its germicidal value is almost negligible, and it finds little practical use.

To summarize the conclusions that may be drawn from the foregoing we find:

First, formaldehyde, when used under conditions in which the gas acts in an aqueous solution, is an efficient disinfectant, probably the only generally applicable fumigant.

Second, mercuric iodide combined with potassium iodide is an exceptionally valuable germicide for the skin and for disinfecting surgical instruments and vessels. Its germicidal value is 5,000 times as high as that of phenol.

Third, lime water freshly slaked from good stone lime has high germicidal value, but its objectionable features both in itself and in its action on other disinfectants makes its use more or less detrimental.

Fourth, soap can be considered valuable only as an aid to disinfection or as a vehicle for an active agent. Most disinfectants with the exception of mercuric-potassium iodide add little to the efficiency of soap.

Fifth, alcohol as commonly used, *i.e.*, on the skin previously washed with soap and water, is highly efficient, in percentages of 30 or over.

Sixth, a coal-tar disinfectant carefully standardized by one of the accepted methods is valuable in proportion to its phenol coefficient and is less affected than some others by the factors which tend to inhibit the value of a germicide.

Seventh, pine oil disinfectants and other special products require as careful standardization as the coal-tar disinfectants. For safe use the dilution should not exceed a number representing the result of the phenol coefficient, times the dilution of pure carbolic acid which would be used with confidence under similar conditions.

Eight, certain of the aniline dyes give promise of practical value in wounds and as internal antiseptics because of having a selective action toward certain microörganisms and because of their low toxicity.

The old dependable germicides, mercuric chloride, phenol, iodine, hydrogen peroxide, will never be displaced, but undoubtedly their field of usefulness will become more and more restricted. The trend of future research in the field of disinfection leads toward the development of specific germicidal agents.

Appendix.

Protocol 1. From original notes.

Silver compound. Tested by Hygienic Laboratory Method.

	Medium Adjusted to 1.5 acidity.	
Dilutions.	2½ *min.*	5 *min.*
1-25	—	—
50	—	—
75	—	—
100	—	—
150	—	—
200	—	—
250	—	—
300	—	—
400	—	—
500	—	—
600	+	—

	Medium not adjusted—acidity 0.9.	
	2½ *min.*	15 *min.*
1-25	—	—
50	+	+
75	+	+
100	+	+
150	+	+
200	+	+
250	+	+
300	+	+
400	+	+
500	+	+

Protocol 2. From original notes.

Formaldehyde gas generated in a space of 800,000 cc. capacity.

54 cc. failed to kill bedbugs and roaches.

16 cc. failed to kill flies and moths.

These quantities exceed the amount necessary for efficient fumigation, which is 13 cc. for this space. The experiments with bedbugs and roaches were continued with increasing amounts to determine if any quantity is toxic in vapor form.

The solution is said to be more or less toxic to flies when ingested, but is by no means a specific poison when so used.

Protocol 3. From Bul. No. 27, Hygienic Laboratory.

Experiment 4.

Formalin used	600 cc.
Permanganate used	300 gm.
Capacity of room	2,000 cu. ft.
Yield of formaldehyde	33 %
Temperature	77° F.
Relative humidity	72 %

Results	5	10	15	20	30	45	60
B. pyocyaneus	+	+	—	—	—	—	—
B. coli communis	+	—	—	—	—		
B. dysenteriæ	+	+	—	—	—	—	—
B. subtilis	+	+	+	+	+	+	—

Experiment 6.

Formalin used	600 cc.
Permanganate used	300 gm.
Capacity of room	2,000 cu. ft.
Yield of formaldehyde	37.7 %
Temperature	71° F.
Relative humidity	45 %

Results	5	10	20	45	60	90	120
B. pyocyaneus	+	+	+	+	—	—	—
B. coli communis	+	+	+	—	—		—
B. dysenteriæ	+	+	+	+	—	—	—
B. subtilis	+	+	+	+	+	+	+

Difference due to percentage of humidity.

Protocol 4. From original notes.

Germicidal tests of samples of Castile soap. Sample 1-6 inclusive were practically neutral. Sample No. 8 was distinctly more alkaline than No. 7, and both contained decidedly more alkali than the others.

Sample.	*Coefficient.*
1	.05
2	less than .05
3	less than .05
4	.07
5	.05
6	.05
7	.18
8	.26

Adding 1 part KOH to 30 parts of No. 4, the resulting mixture had a coefficient of .3.

Protocol 5. From "Soaps from different glycerides," Hamilton. *J. Ind. & Eng. Chem.*, Aug., 1911.

Test organism—B. typhosus.

+ indicates growth in subculture.
— indicates no growth in subculture.

Glyceride.	*Oil used.*	*Dilution.*	*Result in 5 min.*
Trilaurin	Cocoanut	1-40	—
		1-50	+
Tribrassin	Rape seed..............	1-20	+
Trivalerin	Whale	1-20	+
Trilinolein	Linseed	1-20	+
Triricinolein	Castor	1-20	—
		1-30	+
Tripalmitin	Palm	1-20	+
Triolein	Olive	1-20	—
		1-30	+
	Resin	1-20	+

Protocol 6. From "Germicidal Value of Ethyl Alcohol," Harrington & Walker. *Boston Medical and Surgical Journal,* Vol. 148, 1908, p. 548.

B. typhosus—Dried.

Alcohol	5	10	15	30	45	60 min.
15%	+	+	+	+	+	+
20	+	+	+	+	+	+
25	+	—	—	—	—	—
30	—	—	—	—	—	—
80	—	—	—	—	—	—
85	+	+	+	—	—	—
90	+	+	+	+	+	+
B. typhosus—Moist.						
15	+	+	+	+	+	+
20	+	+	+	+	+	+
25	+	—	—	—	—	—
30	—	—	—	—	—	—
90	—	—	—	—	—	—
99	—	—	—	—	—	—

From original notes.

Ethyl Alcohol	*B. typhosus—moist.* 1	2	3	4	5 min.
25	+	+	+	+	+
30	+	+	+	+	+
40	—	—	—	—	—
85	—	—	—	—	—
90	+	—	—	—	—

Protocol 7. Phenol coefficients claimed for a commercial product.

Toward	B. pestis	40
	B. diphtheria	30
	B. entericus	20
	B. coli	17
	Cholera	42

From original notes.

Coefficients of two disinfectants based on two different organisms.

	B. typhosus.	*B. pyocyaneus.*
Disinfectant 1	4	2
" 2	16	4

Protocol 8. From original notes.

Phenols separated from coal-tar oil and redistilled further to separate at each 10° C. or at such points as to obtain sufficient for germicidal test.

Emulsive disinfectants containing 78 per cent. of oil were made from each.

Boiling points.	*Coefficients.*
200°-210° C.	5.5
210°-220° C.	8.7
220°-230° C.	14.5
230°-240° C.	18.1
240°-280° C.	24.

The entire phenols made into a similar product had a coefficient of 17.

Ordinary cresylic acid with boiling points of 185°–205° has a coefficient of 4-5.

Protocol 9. From original notes.

Phenol in aqueous solution.

	2½ min.	*15 min.*
1- 90	—	—
1-100	+	—
1-110	+	—
1-120	+	+

Phenol molecularly combined with caustic soda.

1-20	—	—
1-40	—	—
50	+	—
60	+	+
80	+	+

Phenol molecularly combined with lime.

1-20	—	—
40	+	+
60	+	+
80	+	+

Protocol 10. From original notes.

Cresylic acid and soap.

[Cresol Compound U. S. P.] containing 50 per cent. cresols.

Dilutions.	*2½ min.*	*15 min.*
1-180	—	—
200	+	—
220	+	—
240	+	+

Cresylic acid combined molecularly with lime.

Dilutions based on cresol content.

1-175	—	—
200	—	—
250	+	—
300	+	+
350	+	+

Cresylic acid combined molecularly with caustic soda.

1-160	—	—
200	+	—
240	+	+
280	+	+

Coefficients based on the cresol content.

1st approximately	4
2nd "	2.4
3rd "	2

Protocol 11. From original notes.

Germicidal assay of freshly slaked lime.

Dilutions based on the lime (CaO) used.

Dilutions.	*15*	*30*	*45*	*60 min.*
1-1,000	—	—	—	—
1,500	—	—	—	—
2,000	—	—	—	—
2,500	+	—	—	—
3,000	+	+	+	+
4,000	+	+	+	+
Phenol dilutions.				
1-120	—	—	—	—
140	+	—	—	—
160	+	+	+	—
180	+	+	+	+

Coefficient about 20.

Protocol 12. From original notes.

Disinfectant No. A, about 80 per cent. Cresylic Acid [Cresylic Acid 8 cc., Soft Soap 2 gm.]

Disinfectant No. B, about 50 per cent. Cresylic Acid [Cresylic Acid 5 cc., Soft Soap 5 gm.]

HYGIENIC LABORATORY METHOD.

Dilutions.	*Results.*			
	2½ *min.*		15 *min.*	
	A	B	A	B
300	—	—		
400	—	+		
450	—	+		
500	—	+		
550	—			—
600	—			—
650	+		—	+
700	+		—	+
750			—	+
800			—	
850			+	

Phenol.	2½ *min.*	15 *min.*
90	—	
100	+	
110		—
120		+

Coefficient A 6.9.
B 4.6.

When calculated to the cresylic acid on the basis the amount contained in each ,

Coefficient A–8.6.
B–9.2.

Protocol 13. From original notes.

Germicidal Assay—Hygienic Laboratory Method.

Sample of disinfectant that has been exposed to the light August 28-September 28. (1) compared with the original sample (2).

		2½	15 *min.*			2½	15 *min.*
	1-1,200	—			1-1,200	—	
	1,400	+			1,400	—	
(1)	1,600	+	—	(2)	1,600	—	—
	1,800	+	—		1,800	+	—
	2,000		—		2,000		—
	2,200		+		2,200		+
	1-1,200	—			1-1,200	—	
	1,400	—			1,400	—	
	1,600	+	—		1,600	—	—
	1,800	+	+		1,800	+	—
	2,000		+		2,000		+
	2,200		+		2,200		+
	1-1,400	—	—		1-1,400	—	—
	1,600	+	—		1,600	—	—
	1,800	+	+		1,800	+	—
	2,000	+	+		2,000	+	+
	2,200	+	+		2,200	+	+

Phenol.

1-100	—	—
110	+	—
120	+	—
130	+	+
140	+	+

Coefficient (1) 13.6.
(2) 15.

Protocol 14. From label on package of disinfectant: "Leaves no odor. A powerful antiseptic and germicide of the Naphthalene series."

Inert Ingredients.
Water 23 per cent.
Glycerine 40 per cent.

"Seven times less poisonous and ten times more efficient than carbolic acid."

From results of assay:

Dilutions.	2½	5 *min.*
1-100	—	—
175	+	—
200	+	—
250	+	+
300	+	+
400	+	+
600	+	+
800	+	+
1,000	+	+
Phenol.		
110	—	—
120	+	—

Coefficient 1.6.

Protocol 15. From Public Health Reports, Oct. 8, 1915, p. 3008.

B. typhosus, 20° C.

Phenol.	2½	15 *min.*
80	—	—
90	+	—
100	+	
110	+	—
120	+	+
Pine Oil Disinfectant.		
375	—	—
400	—	—
450	+	—
500	+	+
550	+	+

Coefficient 4.75.

Note growth from 500 dilution in 15 min. subculture.

Protocol 16. From original notes. Pine Oil Disinfectant. From John A. MacKeathan, Fayetteville, N. C.

HYGIENIC LABORATORY METHOD.

Dilutions.	2½	15 *min.*
1st test.		
1-150	+	—
200	+	+
300	+	+
400	+	+
500	+	+
600	+	+
2d test.		
1- 50	—	
75	+	
100	+	+
125	+	+
150	+	+
200	+	+
3d test.		
1- 30	—	
40	—	
50	—	—
75	+	—
100	+	+
125		+
Phenol.		
1-100	—	
110	+	—
120	+	—
130		+

Coefficient .55.

Protocol 17. From original notes.

Chloramine Assay—Hygienic Laboratory Method.

	2½ *min.*	15 *min.*
1,800	—	
2,000	—	
2,200	+	
2,400	+	—
2,600	+	—
5,000		—
6,000		—
8,000		—
10,000		—
12,000		+

Coefficient about 50. This test shows how important a factor time is in testing the value of Chloramine.

Protocol 18. From original notes.

Germicidal test of a mixture of water soluble phenols applicable where freedom from toxicity and irritation combined with high efficiency are essential factors.

Dilution.	*Time and Results.*	
	2½ min.	15 *min.*
1,800	—	
2,000	—	—
2,200	+	—
2,400	+	—
2,600	+	+
Phenol.		
90	—	
100	—	
110	+	—
120		—
130		+

Coefficient 20.

Protocol 19. From original notes.

Germicidal value of a saturated solution of Chloretone in distilled water (about 0.8 per cent.). The solution was inoculated with a culture of *B. typhosus* and sub-cultures taken at 1 minute intervals as follows:

1	2	3	4	5	6	7	8	9	10
+	—	—	—	—	—	—	—	—	—

Protocol 20. From original notes.

Germicidal test of Methyl Violet against *B. diphtheria*. Subcultures made at 1 minute only.

Dilutions.	*1 min. Sub-cultures.*
1,000	—
2,000	—
5,000	—
10,000	—
15,000	—
17,000	—
18,000	+
20,000	+

Test of Gentian Violet under the same conditions.

Dilutions.	*1 min Sub-cultures.*
1,000	+
5,000	+
10,000	+
20,000	+
50,000	+

Test of Malachite Green under same conditions.

Dilutions.	*1 min. Sub-cultures.*
500	—
1,000	—
1,200	—
1,400	+
1,600	+
2,000	+

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CANINE DISTEMPER: REVIEW OF THE WORK OF VARIOUS INVESTIGATORS IN THE ATTEMPT TO ESTABLISH THE PRIMARY ETIOLOGICAL FACTOR.*

N. S. Ferry, Detroit, Mich.

Rabies and distemper are the two oldest, the most destructive and the widest known diseases of the canine race. Just when canine distemper was first described is a question that will probably never be answered. Rabies is said to have been recognized before the time of Aristotle, although rabies and distemper were never truly differentiated until 1809, at which time Jenner clearly demonstrated that the two diseases were distinct entities. Virgil and Aristotle mention a disease, which appears to have occurred as an epizootic during or just before their time, under the name "cynanche;" a clear description of the disease, however, is not given. A disease among the canine race, which raged as an epizootic in Bohemia during the year 1028, was described by Laosson as true distemper. If this is a fact, it is the earliest authentic account of the disease of which we have record. According to Stegman it was observed in Germany in 1697; and Ulloa, in his "Historical Travels in America," mentions observing an eruptive disease among dogs in South America in 1725. It has been said that it was brought into Europe from America soon after the discovery of this country, while others claim that the introduction into Europe was much later—about the middle of the eighteenth century—being carried first to Spain and thence to France, Germany, and finally spreading rapidly to other countries. However, it is an open question as to when and how the disease made its first appearance in Germany and France, as the older French writers considered that the disease was brought into their country from England, while the English editors maintained that it was imported from France into England. This latter contention was probably true, as it was known in France in 1740 and in England not until nearly twenty years later. In Germany it was known in 1748, and in Italy in 1764. In Russia and Siberia it was recognized in 1764 and 1787 respectively, and, in these latter two countries, it is a question of great importance, as the disease works great havoc among the sledge dogs, which are practically the only means of transportation. In Greenland and Iceland the disease has been so disastrous since 1859 as to nearly exterminate the species in those countries.

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*Read before the second annual meeting of the Wisconsin Veterinary Medical Association, Madison, Wis., Jan. 16th to 18th, 1917.

As a rule the disease is not considered one of great economic importance, yet thousands of dollars are lost yearly through the death of many valuable animals, and for that reason, as well as for scientific purposes solely, a large amount of experimental work has been carried on during the past few years to determine, if possible, the principal etiologic factor, and many attempts have been made to place the therapeutics of the disease, both prophylactic and curative, on a scientific basis.

Following the work of Jenner in 1809, who showed us the difference between rabies and distemper, several articles were written assigning to the rôle of the principal etiologic factor many various and curious conditions, but it was not until 1875 that the first microörganism was described as the cause. At this time Semmer published an article in which he claimed he had found a small bacillus in the blood of affected dogs a few hours after death. After this work was brought out, which was really the beginning of the study of the disease along scientific lines, numerous papers were written bearing on the subject of the etiology, and as many different microörganisms were described as there were investigators, but it was not until between 1908 and 1912 that an attempt was carried through apparently successfully to prove the relation of any single organism to the disease by means of experimental inoculation and specific serum reactions. The organism with which this work was done was described first by Ferry (1) (2) in 1910, followed by McGowan (3) (5) (6) in 1911 and by Torrey and Rahe (4) in 1913.

Of the various investigators who published between 1875 and 1910 the following are the most prominent: Krajewski in 1881 described a micrococcus; Mathis in 1887 a diplococcus; Marcone and Meloni in 1886 a coccus similar to the staphylococcus; Jacquot and Legrain in 1890 a motile bacillus; Millais in 1890 a long bacillus; Schantyr in 1892 divided distemper into three distinct diseases and described three organisms, one for each disease; Jensen in 1898 described a streptococcus as the cause of the pneumonia so frequently seen as a complication; Valerio in 1896 a bacillus that produced gas; Babes and Barzanesco in 1897 a bacillus, and Petropawlowsky in 1897 also a bacillus similar to the colon bacillus; Taty and Jacquin in 1898 a diplococcus; Copeman in 1900 a small bacillus with which he made a vaccine said to produce favorable results; Lignieres and Phisalix in 1900 and 1901 described a long bacillus with which they also made a vaccine and a serum.

In 1905 Carré (14) (15) described some experiments carried on by him which convinced him of the existence of a filterable virus. This was later confirmed by Lignieres, and, as a result, the filterable virus or ultramicroscopic theory is still held by a large number of writers.

The microörganism first described by Ferry and named by him "Bacillus bronchisepticus" (7), was isolated up to the time of his second publication from 97 dogs at autopsy. In 68 cases it was isolated in pure culture, either from the respiratory tract or blood. The organism was found in 12 out of 42 blood cultures. From 29 laboratory dogs with symptoms of distemper M'Gowan found the same organism in 27 cases; being isolated in most cases from the mucous membranes of the nose. The organism was not found by him in blood cultures. Torrey found the organism in 65 out of 80 cases; from the liver in 32% of the cases, from the kidney in 16%, and the blood in 6%.

By means of inoculation experiments both Ferry and Torrey were successful in producing the disease in normal dogs, Ferry by the tracheal route and Torrey by the nasal.

The relationship of the organism to distemper has also been shown by means of agglutination, complement fixation and other specific tests, so that proof as regards the specificity of the organism is not lacking.

It is also of interest to know that this microörganism is known to produce a disease somewhat similar to distemper in other animals, such as the rabbit, guinea-pig, cat, monkey, ferret, and likewise in a few cases in the human subject. M'Gowan in 1911 published his findings in the dog, cat, rabbit and guinea-pig, while Ferry (7) (8) (9) (10) in 1912 corroborated this and added the ferret and monkey to the list. The organism has been isolated from the human subject by M'Gowan, Ferry and the New York Board of Health Laboratory. The organism has also been isolated from the guinea-pig by Smith (21) in 1914, Holman (23) in 1916, and Rhea (22) in the dog in 1915.

During the past year, also, a large amount of work has been carried on by Kolmer and Reichel (16) (17) and their associates, to determine, as far as possible, the exact status of the B. bronchisepticus, and, while two papers have already been published, it is understood that others (18) are to follow and at present no definite conclusions are available.

Stimulated by the results of some work of Mallory (25) (26) (27) and his associates in Boston in 1912 and 1913, who suggested that B. bronchisepticus and B. pertussis appeared to be rather closely associated, Ferry (12) (13) and his associates, in a series of papers to be published later, have shown that, while the cultural characteristics and some of the serological reactions are, in many respects, identical, giving the organisms the appearance of being closely related, yet enough difference can be detected to prove conclusively that they are distinct.

It has also been suggested by Horn and Victors (19) of San Francisco, as a result of some comparative tests between B. bronchisepticus and Coccobacillus fetidus ozenæ (Perez) that these two organisms are "indisputably in the same class" and that "the advanced hypothesis that the infection of fetid ozena is carried by dogs, presents an interesting complex." This is a phase of the question that should be determined as soon as possible. Work is being carried on at the present time by the author with that end in view (20).

The contention relative to the filterability of the virus of distemper must also be settled before the question as to the specificity of B. bronchisepticus is solved to the entire satisfaction of all authors. Carré, Lignieres and the majority of writers on the subject, up to the last few years, have argued, as a result of the work by Carré, that the virus of distemper is filterable or ultramicroscopic. On the other hand, a large number of observers, as a result of the experiments of Ferry, M'Gowan, Torrey and others, believe that B. bronchisepticus is indisputably the cause of the disease. It is also of interest to know that Ferry (11) has proved, by means of a series of experiments, that B. bronchisepticus is small enough to readily pass through the pores of both the Berkefeld and Pasteur filters, and therefore is a filterable virus. Wherry (24) claims to have previously filtered this organism. It was also discovered that the less pressure was used the more often would the organism be found in the filtrate. Accordingly then, as Ferry has stated, "If B. bronchisepticus is the cause of canine distemper, then the experiments corroborate the work of Carré. If the work of Carré was correct, and if the causative agent of canine distemper is a filterable virus, then the experiments point very conclusively to B. bronchisepticus and confirm the findings of the three previously mentioned investigators."

In order to further substantiate the position given to B. bronchisepticus, Ferry and also Torrey and Rahe carried on a relatively large number of prophylactic inoculations with suspensions of the dead organisms and found, in a large percentage of the cases, that a true protection had been established in the inoculated dogs, and that the controls, in practically all cases, rapidly succumbed to the ravages of the disease.

Based on the results of the experiments of these observers, bacterial vaccines containing this organism are now listed by several reputable commercial houses. When used with proper precaution, extremely gratifying results, both prophylactic and curative, have been reported. As regards the prophylactic use of the vaccines, failures can be satisfactorily explained on the ground that these vaccines are still practically in the experimental stage, and there is no doubt that improved methods of preparation as well as a more satisfactory technic of administration will insure better results in the future. Often the dogs are exposed too soon after treatment and, as their resistance has been lowered, as a result of the injection of the antigen, they are more liable to infection than a normal dog under the same conditions. Perhaps in some cases the doses are too large, and in others too small.

These questions can only be answered by experience. When using the vaccine for therapeutic or curative purposes, however, it must be remembered that the condition is that of an acute general infection, complicated with severe secondary infections by the time they are first called to the attention of the veterinarian—in which case vaccines would have very little chance of producing beneficial results.

In order to overcome this situation, a situation often attributed to the vaccine itself, an antidistemper serum has been prepared which offers more hope than vaccines for these acute conditions, and which bids fair to entirely replace them, in the future, as a curative agent. A serum, for prophylatic purposes, is of little value unless used simultaneously with the vaccine.

REFERENCES.

1. Ferry: "A Preliminary Report of the Bacterial Findings in Canine Distemper," Am. Vet. Rev., 1910, vol. xxxvii, p. 499.

2. Ferry: "Etiology of Canine Distemper," Jour. Infect. Dis., 1911, vol. viii, p. 399.

3. M'Gowan: "Some Observations on a Laboratory Epidemic, principally among Dogs and Cats, in which the Animals affected presented the Symptoms of the Disease called Distemper," Jour. Path. and Bact., 1911, vol. xv, p. 372.

4. Torrey and Rahe: "Studies in Canine Distemper," Jour. Med. Res., 1913, vol. xxvii, p. 291.

5. M'Gowan: "On an Epidemic among Cats, supervening on and simulating Distemper," Jour. Path. and Bact., 1911, vol. xvi, p. 257.

6. M'Gowan: "Some Observations on the Clinical Symptoms, Prophylaxis and Treatment of Distemper," The Vet. Jour., 1912, vol. lxviii, p. 7.

7. Ferry: "Further Studies on the Bacillus Bronchicanis, the Cause of Canine Distemper," Am. Vet. Rev., 1912, vol. xli, p. 77.

8. Ferry: "Bacillus Bronchisepticus (bronchicanis): The Cause of Distemper in Dogs and a Similar Disease in other Animals," The Vet. Jour., 1912, vol. lxviii, p. 376.

9. Ferry: "Bacillus Bronchisepticus: Its Relation to Canine Distemper," Am. Vet. Rev., 1912, vol. xliii, p. 16.

10. Ferry: "Bacteriology and Control of Acute Infections in Laboratory Animals," The Jour. of Path. and Bact., 1914, vol. xviii, p. 445.

11. Ferry: "The Filterability of Bacillus Bronchisepticus: With an argument for a Uniform Method of Filtration," The Jour. of Path. and Bact., 1915, vol. xix, p. 488.

12. Ferry and Noble: "Studies Relative to the apparent close Relationship between Bact. Pertussis and B. Bronchisepticus: Cultural, Agglutination and Absorption Reactions. To be published.

13. Ferry and Klix: "Studies Relative to the apparent close Relationship between Bact. Pertussis and B. Bronchisepticus: Complement Fixation Tests." To be published.

14. Carré: "La Maladie des Chiens," Biol. Soc. de Med. Vet., 1905, vol. lix, p. 148.

15. Carré: "La Maladie des Chienes-Etiologic at Vaccination," Tenth International Veterinary Congress, London, 1914.

16. Kolmer, Harkins and Reichel: "A Cutaneous Reaction in Canine Distemper," The Jour. of Immun., 1916, vol. i, No. 5.

17. Kolmer, Matsunami and Harkins: "The Relation of the Bronchisepticin Skin Reaction to Immunity in Canine Distemper Including the Bactericidal Action of Dog Serum for B. Bronchisepticus," The Jour. of Immun., 1916, vol. i, No. 5.

18. Reichel, Kolmer, Harkins and Heist: Studies in Canine Distemper. To be published.

19. Horn and Victors: "A Contribution to the Bacteriology of the so-called Coccobacillus Fetidus Ozenæ (Perez), with Additional Notes on the Treatment of Clinical Ozena by means of Polyvalent Vaccines Made from the Same Organism," Annals of Ot., Rhin. and Laryn., 1916, vol. xxv, No. 2.

20. Ferry and Noble: "Ozena and Distemper: Comparative Study of Coccobacillus Foetidus Ozenæ (Perez) and B. Bronchisepticus. To be published.

21. Smith: "Some Bacteriological and Environmental Factors in the Pneumonias of Lower Animals with Special Reference to the Guinea Pig," Jour. of Med. Res., 1914, vol. xxix, p. 291.

22. Rhea: "The Comparative Pathology of the Tracheal and Bronchial Lesions Produced in Man by B. Pertussis (Whooping-cough) and Those Produced in Dogs by B. Bronchisepticus (Canine Distemper)," Jour. of Med. Res., 1915, vol. xxxii, p. 471.

23. Holman: "Spontaneous Infections in the Guinea Pig," Jour. of Med. Res., 1915, vol. xxxv, p. 151.

24. Wherry: "Remarks on the Filterability of B. Bronchisepticus," Jour. of Inf. Dis., 1916, vol. xix, p. 304.

25. Mallory and Horner: "Pertussis: The Histological Lesions in the Respiratory Tract," Jour. of Med. Res., 1912, vol. xxvii, No. 2.

26. Mallory: "The Pathological Lesions of Whooping Cough," Boston Med. and Surg. Jour., 1913, vol. clxix, No. 2.

27. Mallory, Horner and Henderson: "The Relation of Borde-Gengou Bacillus to the Lesions of Whooping Cough," Jour. of Med. Res., 1913, vol. xxvii, No. 4.

For further references see articles by M'Gowan, Torrey and Ferry.

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THE FREQUENCY AND SIGNIFICANCE OF DYSFUNCTION OF THE INTERNAL SECRETORY SYSTEM IN THE FEEBLEMINDED.*

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and H. A. Haynes, M.D., Lapeer, Mich.

At the Michigan Home and Training School, a State institution for the care of the feebleminded, 1,134 inmates have been examined for indications of abnormal functioning of the internal secreting organs. The population of this institution, 1,260, is so diversified as to age and degrees and types of mental deficiency as to accord ample material for quantitative conclusions. The following report is the result of this survey. The cases are not selected either as to diagnosis or treatment. The technical evidences from this series of examinations that serve as the foundation of the conception of the nature of feeblemindedness here expressed are too extensive to be included. Essential points along this line will be grouped in more specialized subsequent reports.

The recent impetus of problems of internal secretion has resulted in activity directed more especially to the clinical aspects of glandular dysfunction. For many years only certain extreme clinical types were associated with glandular irregularity. Upon these infrequently encountered types, exhibiting a totality of manifestations, medical thought was so closely focused that there has been scant recognition of the many gradations between these complete types and normality. Very slowly have we become aware that the protean symptoms of these milder, intermediate glandular disturbances may be determined with reasonable precision in an integral part of the morbid states that come to the attention of the physician. Following the realization of the number of individuals in whose pathological condition a glandular element may be established, there have accrued much additional acceptable evidence indicative of an endocrine factor in widely diversified perversions of growth and development, of nutrition and elimination, of sex differentiation and maturation, of mentality, and of temperament. The correlations existing between feeblemindedness and vegetative glandular dysfunction are, however, but meagrely delineated. This in some measure is attributable to the circumstances that at the time of this renaissance in endocrinology the close attention of those most familiar with the problems of feeblemindedness has been directed to investigations of the intellectual limitations of the feebleminded and the sociological and economical aspects of feeblemindedness.

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*Paper read before the American Association for the Study of the Feebleminded, Indianapolis, May, 1916.

If all life activities are arranged in strata on the basis of phylogenetic progressions, three such levels mark the essential gradations: the level of vegetative activity, the level of reflex activity, and the level of psychic activity. Manifestly in these terms feeblemindedness represents an inadequacy of functioning on the normal psychic level concomitant to inadequacy of functioning on the vegetative level.

The essence of feeblemindedness lies in organic defect, morphogenetic or chemical, having origin in heredity or in embryonic or postnatal malconditions, involving the organism as a whole or through elective action, specific systems or organs.

It is duly recognized that the system of internal secreting glands is but one and not the exclusive source of substances influencing the diversified growth—differentiation processes. However, this endocrine vegetative unit, together with the integrating vegetative nervous system, is so essentially far-reaching that growth and development of the entire body can only proceed along normal lines under conditions of adequately normal functioning of these vegetative organs. Stimulus for the conception of internal secretory anomalies as a causative factor of certain mental deficiencies arises from the frequency of simultaneous occurrence of feeblemindedness and developmental defects of a character known to be influenced by the internal secretory system. If comparison is made between the anomalies of body activity, the outgrowth of known endocrinous dysfunction and impaired body activity demonstrably associated with feeblemindedness, the two in many respects coincide. However, the full measure of frequency and significance of these pathogenic relations remains undetermined and their investigation becomes an obvious duty.

THE VEGETATIVE GLANDULAR SYSTEM—THE VEGETATIVE NERVOUS SYSTEM.

Pathogenesis of glandular diseases.—It is deemed neither necessary nor profitable that this report should cover an exhaustive delineation of the specific individual glandular functions. The entire group of organs is evaluated as a unified system with interlocking functions. Although of different embryonic origins and having peculiar morphogeneses and spatial relations, these organs are intimately correlated one with the other and to a less degree with all other organs and tissues of the body. This ready far-reaching contact is affected and balanced in part through chemical correlations resulting from the exciting and restraining influence on cell dynamics induced by the glandular elaborations—the hormones; in part also these activities are mediated by the vegetative nervous system. The internal secretory organs are themselves vegetative, and although terminology specifies the "vegetative glandular system," and the "vegetative nervous system," functionally the two are not sharply demarcated. The vegetative nervous system is composed of two antagonistic nervous mechanisms: the sympathetic and the autonomic systems. Certain substances are elective in their action upon these antagonistic factors, and through this pharmacodiagnosis the state of responsiveness of these components under various morbid conditions is quantitatively determinable.

These several mutually influenced elements in the vegetative system integrate the physicochemical activities of the body. Such activities, however, represent only one level in life processes; higher are the sensorimotor (reflex) and psychic levels. In the processes of the integration and in the functioning on these higher levels, the vegetative level is brought into intimacy of reciprocal relations. Thus the organism is not composed of isolated autonomies, but of interrelated systems so integrated as to make for a unified total organism.

The normal unfolding of the various growth development differentiation processes betokened at the conjunction of the male and female sex cells, the consummation of which is distributed throughout intrauterine and postnatal life, is dependent in full measure upon adequately functioning vegetative organs. However, notwithstanding this essentiality to the integrity of the entire organism, these organs are themselves subject to disease, to trauma, to a malheredity, to the influence of deleterious agents, and concomitant thereto the whole body is impaired. The etiology of glandular diseases is both diverse and obscure and the factors of incidence here included must be accepted as only partial. Heredity as a prime etiological factor in glandular disease is substantiated by a mass of material suggesting a genetic relation between glandular disturbances, but proof is lacking that these disorders in large numbers conform to Mendelian formulas. It is demonstrable that glandular instability may recur from generation to generation in certain families, but conflicts arise relative to the true inheritance of the condition when it is observed that in the offspring a gland is involved different from that involved in the parent. For example, when the heredity of children of hypopituitary involvement is investigated an appreciable number of the parents and grandparents prove to be cases of hypothyroidism. This suggests that the inheritable factor, rather than regularly being a specific gland abnormality, may be an anomaly of the general vegetative system that may assert itself in any element of that system, especially in the vegetative nervous system.

"Goitre families" are of such frequent occurrence as to attract the attention of the laity; of less frequency, but well authenticated, are familial tendencies to diabetes, ovarian dysfunction, pituitary disease, excessive obesity, or excessive height. Timme reports three generations of glandular disease beginning in 1850 upon the marriage of a "moderate giant" and a diabetic. These are in approximate accordance with Mendelian laws. Among the cases of the present report there are three family groups presenting identical glandular lesions. The inheritance of glandular disease in certain instances involves the much debated question of the inheritance of acquired characteristics. It is noteworthy that endocrinopathies arising from some environmental condition —scarlet fever, trauma, syphilis, neoplasm—may reappear in the offspring. These do not constitute a true inheritance, but rather grow out of lack of homostimulation in intrauterine life, a matter to be again considered in later articles. Such manifestations suggest that the internal secretory system may be a factor in the mechanism of inheritance and thus of far-reaching significance in practical eugenics. The whole matter of glandular inheritance may not be finally judged on data now available, but already the inference is that from an ancestry of vegetative irregularity expressed by glandular dysfunction, we may expect offspring also possessing vegetative irregularities.

Apart from inheritance other prenatal conditions may engender organic deviation. As yet no blastophthoric agent has been determined whose activity is electively directed toward the endocrine system. This system is, however, involved in any generalized blastophthora. The intrauterine life, however, is a

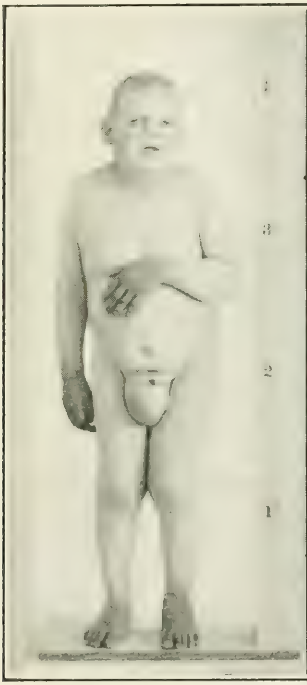


Fig. 1.—Type of glandular disease in the feebleminded. B. M. Chronological age, fifteen years. Mental age, 2.4. (Binet.) Hypopituitarism, hypothyroidism, and hypogonadism.

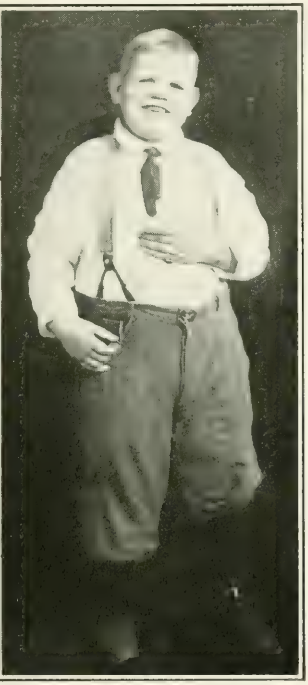


Fig. 2.—Same case as Fig. 1, after two months of glandular therapy. Distinct improvement in both physical and mental condition.

period teeming with opportunity for developmental deviation in which the glandular system may be an especial factor. The implantation of the embryo in the decidual wall of the uterus is dependent upon normality of glandular function. Faulty implantation is regarded by Mall as the source of many physical defects. The developing embryo is bathed in a blood containing nutritive, stimulating, regulating, and often deleterious substances. The growth stimuli of glandular origin are necessarily at first derived from the mother's glandular system. Very early, however, the newly developed fetal glands become functional. McCord has demonstrated a functioning of the pituitary and suprarenal glands within the second month of fetal life. The development of these fetal endocrine glands is influenced by the glandular system of the mother. Just as in the early postnatal life of a child with a glandular lesion, *e.g.,* hypothyroidism, the exhibition of glandular treatment may establish a normal adequately functioning of the deficient gland and treatment becomes no longer necessary, so in the embryo its glandular system appears to be influenced by the homostimulative action of the maternal glandular system. The correlations between the several units of the maternal glandular system is extended to embrace the fetal glandular system. In the mother a defect in any gland tending secondarily to involve others of the maternal system in a pluriglandular lesion may be expected to involve also the fetal glandular system.

In the larval developmental period of lower animal forms, which is only remotely comparable with intrauterine life, the metamorphosis may be influenced almost at will as to rate and character by feeding with endocrine derivatives. Thyroid gland tissues added to the living water of frog tadpoles will bring about the transformation from the tadpole stage to the frog stage within one week, whereas without thyroid feeding the natural transformation would consume three to five months. This striking phenomenon is attributable to the premature differentiation of organs and tissues from thyroid stimulation. Such rapidly formed organs are always imperfect and the tadpoles do not usually survive. Similarly thymus tissue added to the living water of tadpoles retards differentiation. When tadpoles fed upon thymus tissue reach the period for normal transformation into frogs, no differentiation occurs. Such tadpoles continue to grow larger in size without the development of frog characteristics.

Toxic agents that under pathological conditions exist in the human—acetone, butyric acid, histamine—determine gross developmental defects in lower life forms. More elaborate experiments may allow the inference of a similar deleterious influence of such toxic bodies on the developing human. Histamine may prove of special significance, as it represents a type of amine

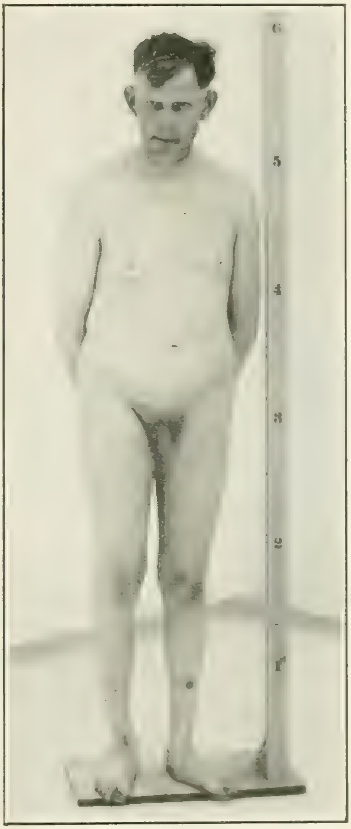


FIG. 3.—Type of glandular disease in the feebleminded. G. D. Chronological age, forty-four years. Mental age, five. (Binet.) Primary lesion hyperpituitarism with associated hypersexual activity, which led to castration, with the subsequent development of hypopituitarism.

arising from intestinal putrefaction or from throat and mouth infections. In the feebleminded of low type, pyorrheas of appalling degree and frequency are encountered. Reasonable definiteness associates such mouth and throat conditions with the origin of glandular disease.

Embryophthora in the human is rarely considered except in relation to such conditions as syphilis, tuberculosis, typhoid fever, and alcoholism. The acuter infections and toxic processes leave so few persistent stigmata indicative of parental pathology that the inference is that the delicate developing organism likewise is unscathed. This assumption is many times apparently borne out when at birth the child is declared to be normal. However, potential normality at birth is no guarantee of its permanency throughout life.

In this recital of possible departures from normal intrauterine life, the glandular system may be involved electively or involved only in the general body impairment from conditions acting alike on all tissues. However, the same degree of impairment involving a gland and, for example, an ear or a muscle is of far greater consequence in the case of the gland, for upon its proper functioning the continued development of the whole organism is dependent.

As a result of these prenatal conditions, inheritance, blastophthora, and embryophthora, we may anticipate an appreciable number of births of individuals evincing various degrees of actual glandular disease or potential ready exhaustibility of the glandular system which succumbs to a trivial postnatal etiology. This postnatal etiology includes: 1, Infectious processes and toxins; 2, neoplasms and cysts; 3, trauma; 4, spatial abnormalities, such as pressure on the pituitary from hydrocephalus; 5, added strains incident to various periods of glandular functional readjustment, *e.g.*, puberty, climacterium; 6, abnormal functional stimuli from the vegetative nervous system.

From an etiology purely prenatal or purely postnatal or from groupings of elements from both, we may expect glandular cases in the following groups: 1. Prenatal etiology with abnormalities, anatomic or functional, demonstrable early in embryogenesis: cretinism, multiple glandular hypoplasias. 2. Prenatal etiology with abnormalities lying dormant and development proceeding nearly normal until the advent of some factors of postnatal etiology which in degree may be trivial and insufficient to determine a lesion in a stable glandular system. The cases in these two groups may be monoglandular, but more often are pluriglandular. A monoglandular lesion may remain pure, but more

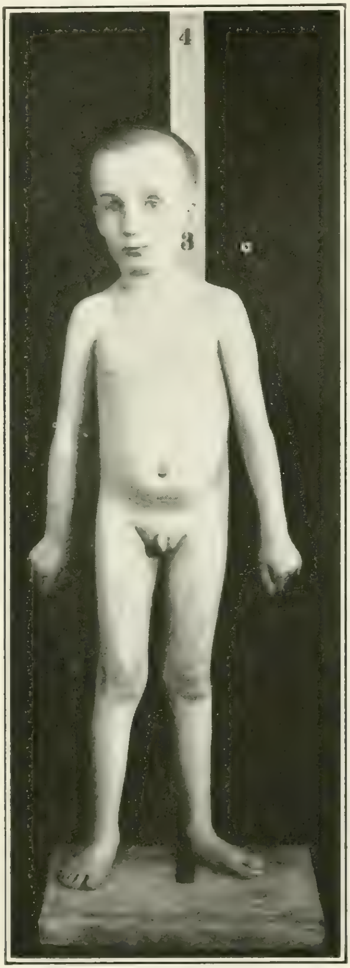


Fig. 4.—Type of glandular disease in the feebleminded. L. H. Chronological age, ten years. Mental age, about two. (Binet.) Hypopituitarism associated with hydrocephalus.

often because of its reciprocal relations becomes pluriglandular in nature even though the secondarily involved glands were primarily normal. 3. Without any factor of prenatal etiology any endocrine gland may become abnormal from postnatal causes, a number of which are grouped above. Primarily these lesions are more frequently monoglandular, but tend through distortion of their balances with other glands to upset the general equilibrium and become pluriglandular.

These states of exalted or of diminished functioning of the several glands and of their pluriglandular combinations are characterized by reasonably definite clinical manifestations. These states are thus recognizable clinical entities.

Glandular diseases and feeblemindedness.—It will already have been perceived that the etiological background from which we may expect certain glandular conditions to arise is also the etiological background from which we may expect certain feeblemindedness to arise. Frequency of simultaneous existence of these conditions growing from a common cause is rational. The coexistence of the two or the exhibition of feeblemindedness subsequent to an already exhibited glandular defect more often does not warrant the immediate assumption that the endocrinic defect is the primary pathogenic factor of the feeblemindedness, or that substitution therapy with derivatives of the corresponding gland from animals should eliminate the mental defect. The visceral, somatic, glandular, and general neural defects observable in the feebleminded and in glandular disease of prenatal origin are relatively fixed in degree, although in their consummation time is an element and their unfolding may be distributed over the normal span of life. Such defects are almost wholly non-irradicable, but to the hypoplasia of the glandular elements there must be attributed an additional significance In the normal postnatal evolution of embryonic structures into the adult forms, there are continuous day to day requirements of the elaborations of these glands necessary for this embryogenesis, and these elaborations withheld determine an increasing defect of the organism as a whole. In this supply of the day to day demands lies the field for substitution therapy, a procedure sharply delimited through the necessary general artificiality of processes and of less promise to the institutional degree of defection than to border-line and preborder-line neuroglandular defectives and to pure glandular defectives.

Up to this point the greater emphasis, because of the probability of greater frequency, has been placed upon conditions the

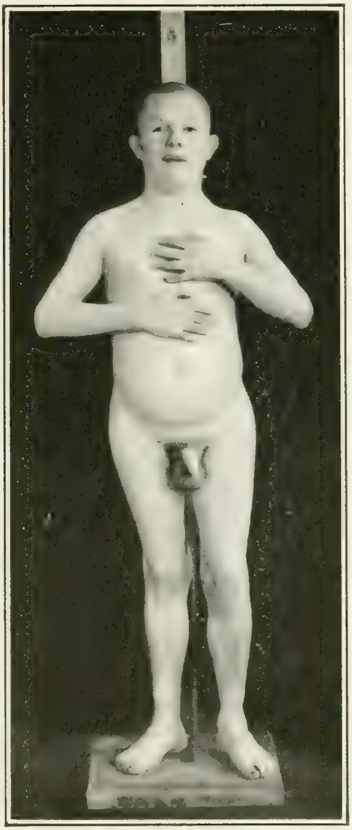


Fig. 5.—Type of glandular disease in the feebleminded. G. S. Chronological age, thirty-four years. Deaf mute. Mild hypothyroidism with distinct physical improvement from glandular therapy.

outgrowth of coöriginating feeblemindedness and glandular defect. In these the glandular anomalies, while not accounting for the primary conditions, are accountable for the increasing defect, both somatic and neural. However, apart from this phase of neuroglandular disease, glandular dysfunctions whose causative agents have in no wise involved the central nervous system may themselves through developmental inhibitions of cortical elements determine a true feeblemindedness. Here again a multiplicity of gradations exist contingent upon the gland or glands involved, the degree of glandular defect, and paramount upon the stage of development at which the malactivity of the gland is exerted. A degree of glandular malfunction acting upon the developing child within its first year of life and producing a true hypoplastic feeblemindedness is likely when acting primarily upon a mature adult to induce scant mental disturbances beyond an apathetic sluggishness.

The convictions here expressed represent the present trend of evidence from an unfinished study of the series of cases now briefly to be described.

Personal examinations of 1,134 feebleminded individuals were made. Of this number 649 were males, 483 females. The range of ages was from six years to the years of normal senility, with a predominance of those in the second and third decades. The degree of mental enfeeblement ranged from a few borderline cases to the lowest grade of idiot. Thirty-seven Mongolian defectives were among the number. The routine of examination, the program of which is not included here, varied with the probability of glandular disease after making a cursory preliminary examination. The limitations we have thrown around this investigation precludes the incorporation in this report of many obvious vegetative malconditions. Cases of pure vegetative nervous system involvement are debarred although such conditions are of widespread occurrence among the low-grade feebleminded. Since this system is an integrator of glandular function its perversions stand at the threshold of glandular irregularity. Of 116 low-grade boys in the first and second decades, ninety-four were found to possess unstable vegetative nervous systems as evidenced in the hypothermia, cyanosis, coldness of acral parts, cardiovascular disturbance, lax joints and tissues, etc. This same group of boys averaged twenty-five pounds underweight for their age. Many were frankly infantile. This report is confined to those vegetative disturbances, demonstrably associated with the symptoms characteristic of glandular dysfunction as manifested in body contour, metabolic deviations, spatial abnormalities, specific chemical reactions, etc.

TABLE I.

Tabulations of Frequency of Endocrinopathology in the Feebleminded.

Cases Examined, 1,134; 21.16% Glandular.	Thyroid				Pituitary			Testicular										
	Enlargement without manifestations.	Hyper.	Hypo.	Total.	Hypo.	Hyper.	Total.	Hypo.	Hyper.	Total.	Ovary.	Pluriglandular.	Parathyroid.	Adrenal.	Total glandular.	Mongolian.	Total number examined.	Percentage endocrine cases.
Females	21	21	6	48	16	1	17	...	...	...	13	16	3	1	98	12	485	22.6
Males	8	10	...	18	23	1	24	42	1	43	...	19	...	1	105	25	649	20.0
Totals	29	31	6	66	39	2	41	42	1	43	13	35	3	2	203	37	1,134	21.16
Female and male: Pecentage of types to total of individual glandular cases	44	47	9	...	95	5	...	97.7	2.3	...	...	...	...	...	...	...	...	...
Female and male: Percentage of cases of individual gland to total of glandular cases	...	...	...	27.5	...	...	17.1	...	...	18.0	5.4	14.6	1.2	.8	...	...	...	...
Female and male: Percentage of cases ot individual gland to total number examined	...	...	...	5.8	...	...	3.6	...	...	3.8	1.15	3.08	.26	.18	17.9	3.2	...	...
Female: Percentage of types to cases of individual gland	43.7	43.7	12.6	...	94.1	5.9	...	...	...	...	...	...	...	...	...	...	...	...
Male: Percentage of types to cases of individual gland	44.5	55.5	...	...	95.9	4.1	...	97.7	2.3	...	...	...	...	...	...	...	...	...
Female: Percentage of cases of individual gland to total of female cases	...	...	...	43.6	...	...	5.4	...	...	...	11.8	14.5	2.7	.9	...	...	...	...
Male: Percentage of cases of individual gland to total of male glandular cases	...	...	...	13.8	...	...	18.4	...	...	33.1	...	14.6	...	.9	...	...	...	...
Female: Percentage of cases of individual gland to total number of females examined	...	...	...	9.9	...	...	3.5	...	...	...	2.6	3.3	.6	.2	...	...	...	...
Male: Percentage of individual gland to total number of males examined	...	...	...	2.7	...	...	3.7	...	...	6.8	...	2.9	...	.15	...	...	...	...

The recognition of the frank glandular cases was a matter of inspection, but in involvements of milder degree and in the more intricate pluriglandular types the criteria on which the diagnosis depends were rendered in many instances inaccessible through the inability of the patients to coöperate with the physician in the more complicated objective tests or to place at his disposal the very valuable subjective evidences. Moreover, many alterations in temperature, the cardiovascular system, blood findings, carbohydrate tolerance, etc., so valuable in the diagnosis of glandular perversions in mentally normal persons, occur in the feebleminded with no other suggestion of glandular disease, with such regularity as to preclude whatever weight such alterations would carry under normal conditions.

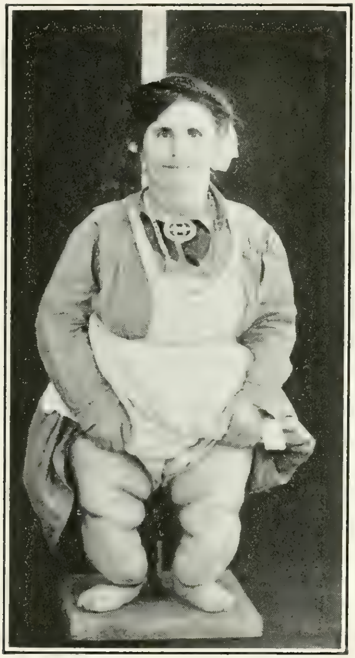


Fig. 6.—Type of glandular disease in the feeble-minded. L. J. Probable quiescent pituitary tumor, hypothyroidism—an inherited glandular condition.

Therefore the numbers included as glandular must be regarded as the minimum number involved which is augmentable through increased insight into the subjectivity of the cases and through more exhaustive objective studies, especially those directed toward an altered metabolism. It is also anticipated that continued study must necessarily increase the number of reported pluriglandular cases at the expense of the monoglandular.

Our findings as to the numbers of glandular cases are grouped in Table I.

These ratios are significant in case they hold true for large numbers of the feebleminded. They indicate that one-fifth of the feebleminded present in varying degrees the indications of malactivity of the internal secretory system. This frequency of involvement has long been anticipated. From earlier established evidence that inefficient endocrine organs may in some degree be quickened to greater activity, supplemented or even replaced by the chemical derivatives obtained from the corresponding glands in animals, there has existed the substantial expectancy that newer developments in glandular therapy would afford means for more successfully treating these arrested developments. However, glandular therapy is so surrounded by sharp natural limitations that a concise, objective summary of its scope of efficacy does not allow any intimation that in feeblemindedness, glandular substitution therapy is generally helpful. Promiscuous glandular treatment of the feebleminded is unprofitable and unwarranted. However, among the feebleminded there occur forms of glandular disturbances that are sufficiently amenable to the influence of glandular therapy that individuals so involved, when early recognized, intelligently and persistently treated, are preserved for reasonably useful lives; whereas if treatment is withheld, the defects become more and more apparent. These statements are more pertinent to the border-line cases than to the type usually to be found in institutions. However, even in institution cases we have been able to establish unquestionable improvements in glandular types. Under conditions of careful quantitative measurements of intellectual capacities prior to and during treatment we have produced distinct physical and mental betterment. The details of diagnosis, treatment, results of treatment, of these cases are reserved for subsequent reports.

SUMMARY.

At the Michigan Home and Training School, a survey has been made of 1,134 feebleminded inmates for evidences of involvement of the internal secretory system in the defects of growth and development in the feebleminded. Of the number examined, 240, or 21.16 per cent., presented the characteristics of various glandular syndromes. From our investigation the following inferences appear justified:

1. Of the glandular cases seen in the feebleminded, heredity stands out as the foremost factor in the etiology.

2. The demonstration of glandular syndromes in the feebleminded does not in itself allow any inference that the feeblemindedness is attributable to the glandular dysfunction. The glandular disease may determine the increasing defect but more often the coexisting feeblemindedness and glandular defect are both the outcome of a common cause.

3. Promiscuous treatment of the feebleminded with glandular derivatives is unprofitable and unwarranted. In established glandular types among the feebleminded, more often glandular treatment is of no distinct and lasting value.

4. In border-line cases of glandular disease with trivial mental inadequacy, glandular therapy may prove of especial value. More often in these cases no true mental deficiency exists, and all manifestations of mental inadequacy are referable to the glandular malfunction. In such cases glandular treatment persistently carried out may be the factor deciding between normality and increasing defects.

103809

Studies from the Research Laboratory
Parke, Davis & Co.
Reprint No. 124, 1917

(Reprinted from *The American Journal of Pharmacy*, April, 1917.)

DIGITALIS THAPSI LIN.

By O. A. Farwell and H. C. Hamilton.
(Departments of Botany and Pharmacology, Parke, Davis & Co., Detroit, Mich.)

Late in 1916, samples of a new drug were offered in America under the name of Spanish digitalis. It has no general resemblance to the official digitalis; indeed, at first glance, it looks more like mullein than anything else, being yellowish-gray or yellowish-green in color.

It has been thought advisable to investigate the histological and pharmacological aspects of the drug, and the following papers are the result. Each author is responsible for his own section of the work.

HISTOLOGY.

By Oliver Atkins Farwell.

The drug consists of broken fragments of leaf (Fig. 4), stems (Fig. 3), and capsules (Fig. 5). Stems slender, terete or somewhat angular, green or purplish, densely covered with gland-tipped, 3-7 celled hairs (Figs. 1 and 2), one-half a millimeter or less in length, velvety not rough. Bases of alternate leaves show a slight, decurrent line (Fig. 3) on the stem; leaves in fragments, rarely entire, 2-3 cm. wide by 3-15 in length narrowly oblong or oblong-lanceolate, gradually tapering to a broad sessile base, margin coarsely denticulate; on the lower surface the midvein is prominent with 4-6 inconspicuous pairs of veins, rugose; both upper and lower surfaces are covered with glandular hairs, but they are not so long as those found upon the stem, soft velvety, not rough. Flowers occasional, cylindrical, upper parts purplish, slightly pubescent externally; when expanded, cylindrical below, contracted just above the ovary and then abruptly expanded into a tube much longer than the usually 5-lobed, slightly 2-lipped limb; stamens 4, didynamous, attached low down on the tube, included; calyx present, glandular, 5-parted, about 1/5 the length of the corolla, segments lanceolate. Capsules ovoid, about 15 mm. in length, greenish and more or less glandular, to pale-brownish or yellowish-green, with only traces of the glandular indument, with fragments of the calyx at the base, on a slender pedicle, 3 cm. or less in length, which is clavate under the capsule; fruit partially separating into two, one-celled, many-seeded sections, opening at the apex on the inner surface by a large pore. Odor, slight; taste, bitter and slightly acrid. This drug is not the official *Digitalis purpurea* Lin. as the leaves are sessile, somewhat decurrent, and of the same color on both sides. It may be the closely related *D. Thapsi* Lin. A cross-section of the upper portion of a stem, 2½ mm. thick, shows a pith 1 mm. in diameter surrounded by a circle of wood (Fig. 6, *D*), 0.3 mm. wide and a bark about .5 mm. The outer bark shows epidermal cells that are small (12-20 microns) with rather thick outer walls covered with a strongly papillate cuticle (Fig. 7, *A*); immediately under the epidermis is a layer (130 microns) of unlignified hypodermal cells 5-8 tiers in depth (Fig. 7, *B*); internally to this is a layer of parenchyma (Fig. 8), of equal depth but composed of fewer, 4-6 tiers of cells; the inner bark (Fig. 6) is made up of a continuous circle of bast (*A*) 130 microns deep and a narrower layer of sieve tissue (*B*). The cells of the pith (*E*) often reach a size of 70 microns in diameter, are slightly lignified or cutinized and non-porous. The stomata (Fig. 9) on the leaf occur in the ratio of about 8 to an area of 150 microns square. The epidermis is essentially the same as for the green bark of the stem. The palisade tissue is one cell in depth; the cells of the sponge tissue are well filled with oil. No crystals were detected. The midrib (Fig. 10) forms a very prominent keel on the dorsal (lower) surface of the leaf. The vascular strand is rather broadly ovate in outline, the phloëm passing almost completely around the xylem. On the ventral side there are several layers of collenchyma and on the dorsal side the greater part of the tissue consists of the water-storage-cells with a layer or two of collenchyma between it and the epidermis.

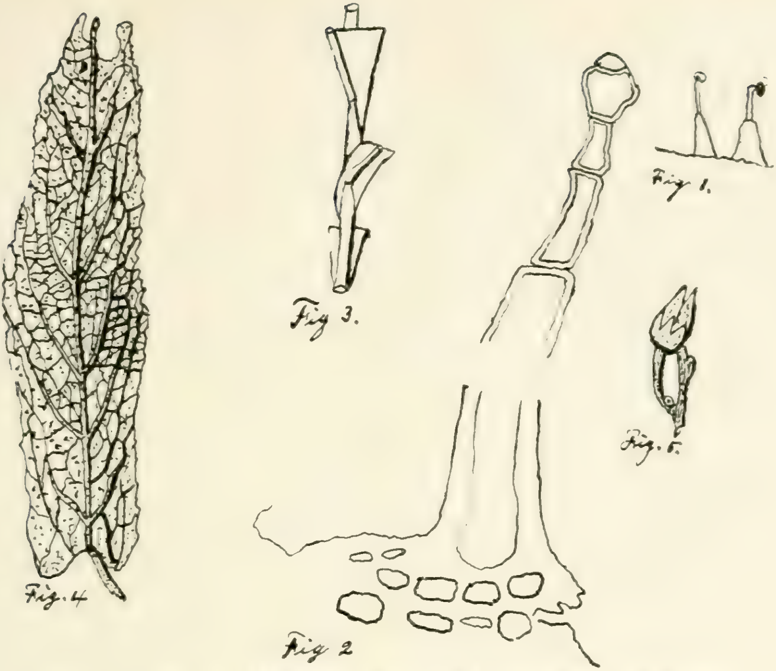


Fig. 1.
Fig. 2
Fig. 3.
Fig. 4
Fig. 5.

FIGS. 1-5. *Digitalis Thapsi* Lin., showing leaf fragments, a capsule and gland-tipped hairs.

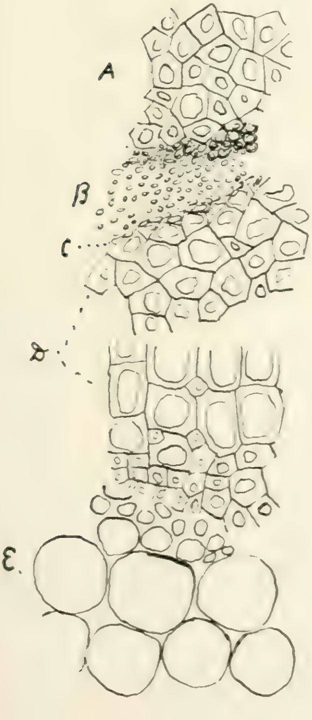
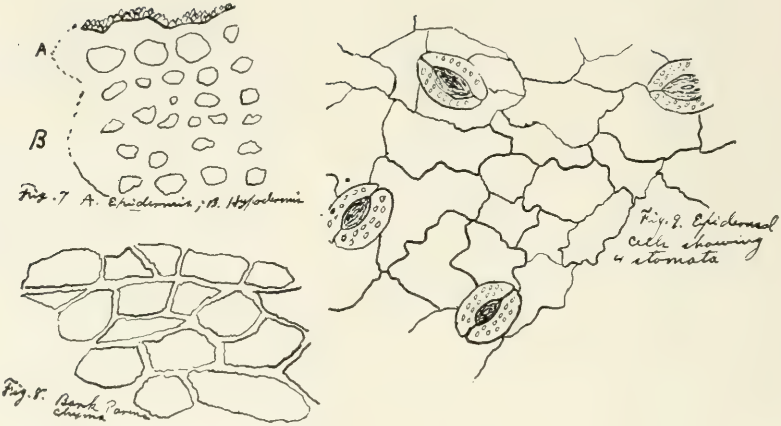


FIG. 6. Transverse section through portion of stem of *Digitalis Thapsi* Lin. *A*, bast fibers; *B*, sieve tissue; *C*, cambium; *D*, woody tissues; *E*, pith.


A
B
Fig. 7 A. Epidermis; B. Hypodermis
Fig. 8. Bark Parenchyma
Fig. 9. Epidermal cells showing 4 stomata

FIGS. 7-9. *Digitalis Thapsi* Lin., showing structure of stem and stomata on leaf.

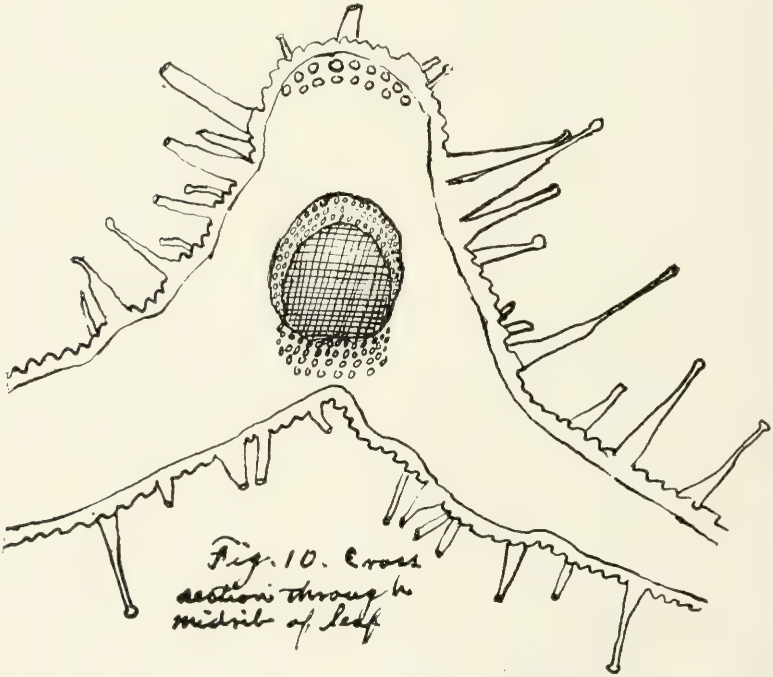


FIG. 10. Cross-section through midrib of leaf of *Digitalis Thapsi* Lin.

PHARMACOLOGIC ACTION.

By Herbert C. Hamilton.

A preliminary investigation of *Digitalis Thapsi* to determine whether it possesses any of the therapeutic properties of the official drug was carried out on frogs by the M. L. D. method for standardization.[1] The drug in tincture form was properly diluted and injected into frogs in gradually decreasing doses until the minimum was found which was sufficiently toxic to kill.

This dose was just one-third that of a similar tincture from an average sample of official drug as shown below. Data of final tests only are here recorded.

Standard Tr. Digitalis.

Wt. of frog.	Dose per gm.	Total dose.
20	.009	.18 alive
20	.010	.20 dead
19	.010	.10 dead
21	.011	.23 dead
22	.011	.23 dead

Tincture from Thapsi Drug.

18	.0025	.23 alive
20	.0030	.30 alive
20	.0035	.35 dead
21	.0040	.42 dead
22	.0045	.49 dead

Dilution 1 in 5.

The above are the final tests showing the comparative toxicities as determined by the minimum lethal doses of the two tinctures, which are:

Standard Tinct. Digitalis	0.010
Tinct. Digitalis Thapsi	0.0035

That the drug belongs to the digitalis series of heart tonics is shown by the fact that in every case the heart was found to have stopped in systole, *i.e.*, with the apex of the heart strongly contracted.

A qualitative test further to confirm the action of the drug was made by dropping the diluted tinctures on the laid-bare frog's heart. The two tinctures were compared as before.

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[1] Houghton and Hamilton, *Amer. Jour. of Pharm.*, October, 1909.

First Experiment.

Frog pithed and stretched out on board.

Normal heart rate.
9:15......20 beats in 20 seconds.
9:16......1 drop tinct. thapsi drug dropped on the laid-bare heart.
9:20......20 beats in 22 seconds.
9:21......20 beats in 23 seconds.
9:24......20 beats in 26 seconds.
9:25......2 drops, same solution.
9:28......20 beats in 30 seconds.
9:30......20 beats in 40 seconds.
9:35......Beats irregular and indistinct.
9:40......Stopped in systole.

Second Experiment.

Frog pithed and fastened to board.

Normal heart rate.
10:30......20 beats in 24 seconds.
10:35......2 drops tr. digitalis U.S.P.
10:37......20 beats in 28 seconds.
10:39......20 beats in 30 seconds.
10:45......20 beats in 30 seconds.
10:46......2 drops, same solution.
10:50......20 beats in 35 seconds.
10:55......20 beats in 40 seconds.
11:00......2 drops, same solution.
11:05......20 beats in 50 seconds.
11:10......20 beats in 55 seconds.
Irregular beats and final stoppage in systole.

Third Experiment.

Normal heart rate.
1:35......20 beats in 20 seconds.
1:40......2 drops thapsi drug diluted, 1 in 3 from the tincture.
1:45......20 beats in 22 seconds.
1:47......20 beats in 23 seconds.
2 drops, same solution.
1:50......20 beats in 28 seconds, very powerful.
1:54......20 beats in 30 seconds.
1:55......2 drops, same solution.
1:57......20 beats in 33 seconds.
2:00......20 beats in 50 seconds.
Very irregular.
2:15......20 beats in 200 seconds, stopped in systole.

These experiments show the typical action of the drug in slowing and strengthening the heart beat to be identical with that of the U. S. P. digitalis. The powerful ventricular contractions were especially noticeable.

The other typical heart-tonic action of digitalis, namely, its pressor effect on the circulatory system, is observed best on an anesthetized dog. The dog is anesthetized with chloretone[2] which affects the system only slightly. These experiments failed to discover this typical effect when thapsi drug was administered, while the tracings obtained after injection of the official drug almost invariably show a distinct rise in blood pressure followed, unless the dose be too large, by a return approximately to normal.

The two drugs were injected one following the other into two dogs. The first dog received first 0.2 Cc. of the tincture (freed from alcohol) of the thapsi drug. The rate was lowered, but no increase of blood pressure could be detected. After the action of the drug was apparently over, about one hour after first injection, 0.5 Cc. Tr. Digitalis, U.S.P., was administered, followed shortly by the characteristic effects of the drug, including a distinct increase in blood pressure. The heart-beats soon became irregular and the dog died.

In another dog the order of dosing was reversed, the official tincture being administered first, followed by an equivalent dose of the thapsi tincture. The effects observed from the first experiment were duplicated in the second, but reversed. The official tincture raised the blood pressure while the thapsi tincture had no effect on the blood pressure, but slowed the heart and the experiment ended by the irregular heart beats and final death of the animal.

While the experiments detailed above are more or less preliminary to a more exhaustive examination of the pharmacologic properties of this variety of digitalis, they are sufficient to show that it possesses at least two of the valuable properties of *Digitalis purpurea*, namely, the effects on the rate and amplitude of the heart beat. They show also that it more nearly resembles strophanthus in having no effect or at least very slight action on the blood pressure[3]—a property which in many cases is a distinct advantage.

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[2] Rowe, *Journal of Pharmacology and Experimental Therapeutics*, vol. 9, 1916.

The observed activity of the drug—a toxicity three times as great as that of the average official variety—is not to be taken as representing the activity of this variety. It represents that of only one sample and does not exceed that of occasional samples of American grown *Digitalis purpurea.* Further work and a number of samples would be required to determine its average activity.

That this drug may become a valuable adjunct to the repertoire of heart tonics is evident from its similarity in action both to the official variety of digitalis and to strophanthus, which latter is becoming more and more generally used in therapy.

A more extended pharmacologic research is planned for the near future.

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[3] Cushney, "Pharmacology and Therapeutics."

103808

Studies from the Research Laboratory
Parke, Davis & Co.
Reprint No. 125, 1917

THE PINEAL GLAND.*

THE PINEAL GLAND'S INFLUENCE UPON GROWTH AND DIFFERENTIATION WITH PARTICULAR REFERENCE TO ITS INFLUENCE UPON PRENATAL DEVELOPMENT.

By Carey Pratt McCord, M.D., Detroit, Mich.

I. INTRODUCTION.

(A cursory review of recent work pertinent to pineal functioning.)

The evidences that link the pineal body with a glandular function are much less definite than for such glandular organs as the thyroid, hypophysis, ovary, and the suprarenals. Doubt is frequently expressed that the pineal body is more than a functionless vestige of what was once, in earlier evolutional stages, a functioning eye. Other observations have led to the contention that the pineal, through metamorphosis, has become a highly specialized tissue that serves the body in a manner comparable with the major members of the endocrinous system.

The purpose of the present paper is to group the essential findings from the recent literature into a concise, unbiased résumé, adequately expressing the status of the pineal body as a functioning organ. To this are added the writer's more recent observations upon the growth of young animals under the influence of pineal materials.

ANATOMY AND EMBRYOLOGY.

(Bibliography numbers 1-57.)

The pineal body (pineal gland, epiphysis, conarium) is situated in the brain just beneath the splenium of the corpus callosum. (Fig. 1.) It lies suspended between the anterior quadrigeminate bodies. The gland is consequently just above the Sylvian aqueduct. The internal cerebral veins lie above and partially encircle the pineal. In the human the pineal is nearly trilateral in shape, in sheep is round, in cattle is oval. The average weight in cattle is .2 gram and in sheep .13 gram. Primarily the pineal is developed as a thin ependymal diverticulum from the diencephalon, extending between the posterior and habenular commissures. At a later stage this diverticulum thickens and encloses some of the adjacent vascular mesoderm to form the mature organ. (Streeter.)

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*Reprinted from the Transactions of the American Gynecological Society, 1917.

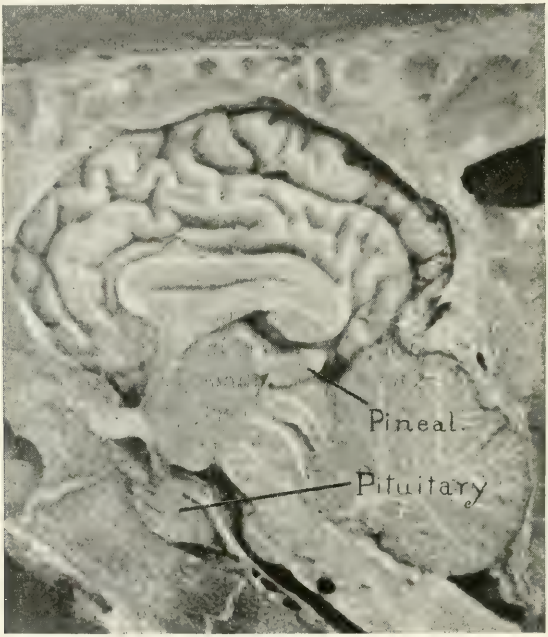

FIG. 1.—Sagittal section of beef brain, showing size, position, and relation of pineal gland.

In those publications cited in the bibliography as pertinent to the anatomy, embryology, and histology of the pineal, the studies have, for the most part, been prosecuted toward establishing (1) the presence of glandular tissue; (2) the presence of contractile tissue supporting the view that the gland is a valve regulating the flow of cerebrospinal fluid; (3) nerve fiber communication between this gland and other parts of the brain; (4) evidence of involution changes in the gland indicating a cessation of function.

These publications may be summarized as indicating: (1) Complete cytologic studies in several species allow the inference that the pineal body is glandular in nature. The glandular elements, however, are few and illy defined. (2) The occasionally demonstrated muscle fibers in the pineal are without significance to pineal function. (3) Nerve fibres and neuroglia are to be found at least in certain animals, but these are probably of trivial import. (4) The gland undergoes involution changes, beginning in the human as early as the seventh year. Involution is pronounced at puberty. The degeneration is, however, not complete and the histologic picture of the adult gland is not such as to remove the possibility of a continued function in adult life.

PINEAL NEOPLASMS AND RESULTING FUNCTIONAL DISTURBANCES.

(Bibliography numbers 100-189.)

Tumors of the pineal are not of frequent occurrence. The total number of authentic cases, with subsequent necropsy findings in some, is not more than 70. These cases have been the source of the greatest information as to the functions of the pineal. In 1898, Heubner described a boy of 4½ years who showed a precocious sexual and somatic growth. The body of this boy was that of a boy of 8 or 9 years. The genitalia corresponded to the proportions found at puberty. The pubic hair was 1 cm. long. A year later at autopsy a teratoma of the pineal was demonstrated. By 1907, Marburg was able to collect 40 histories of such types. He sought to establish a clinical entity for pineal dysfunction. The term "Macrogenitosomia præcox" has subsequently designated this condition. In a more recent paper, Marburg attributes to the condition the following characteristics:

1. General. These include all the usual signs of intracranial pressure, usually secondary to a subsequent internal hydrocephalus.

2. Neighborhood. These for the most part are dependent upon encroachment upon the quadrigeminate bodies, leading to diverse oculomotor paralyses and pupillary disturbances, and encroachment upon the cerebellum, with ataxic manifestations.

3. Constitutional. Under this designation are grouped the manifestations attributable to the derangement of the pineal

glandular function. This constitutional syndrome consists of first, early sexual maturity, evidenced in the enlarged sex organs, pubic hair, general body hair, early change in voice; second, precocious mental development, evidenced in the maturity of thought and speech; third, general body overgrowth to the extent that a child of 5 or 6 years may have the appearance of a child of 11 or 12.

Frankl-Hochwart similarly has summed up the characteristics of this pathologic state. He states, "When one finds in a very young individual, along with the general symptoms of tumor as well as the signs of a lesion of the corpora quadrigemina, abnormal body growth, unusual growth of hair, adiposity, somnolence, premature genital and sexual development, and finally intellectual maturity, one must think of pineal tumor."

Of the 70 cases at the present time available in the literature, only 25 occurred prior to puberty. Because of the pineal involution that occurs by the time of puberty, only in these 25 cases are constitutional manifestations to be anticipated. It is significant that with two exceptions all cases occurred in boys.

Many cases of pineal tumors before puberty manifest none of the signs of precocity of development that are so striking in a few selected cases. A study of the clinical material reveals how little consideration has been given to the possibility of pluriglandular involvement. In fact, in some early cases the necropsy demonstration of a pineal tumor led to the association of all prior metabolic changes to pineal functional perversion. This grew out of the prevalent conception of each endocrine gland as an entity entering into no interrelations with other similar organs. Judging these cases in the light of recent advances in pituitary pathology and physiology, it is difficult to delineate the manifestations of pure pineal derangement from a pluriglandular condition. Cushing has pointed out that from the intracranial alterations attending pineal neoplasms, the hypophyseal functions are readily deflected from the normal.

This infrequent condition in which growth and differentiation into the adult is so deviated from the normal that very young children acquire in part the sexual, mental and somatic characteristics of maturity, has naturally led to diverse attempts to induce such a condition experimentally. Through the extirpation of the pineal, through the feeding of pineal substances to young animals, through the intravenous and subcutaneous administrations of pineal extracts, has information been sought as to the significance of this organ in the body's economy. The outcome of such investigations are described in subsequent paragraphs.

EXTIRPATION OF THE PINEAL GLAND.

(Bibliography numbers 71-78.)

Situated near the center of the brain, the inaccessibility of the pineal has prevented any widespread use of this method. The trauma is necessarily severe, and until the recent reports by Dandy (1915) and Horrax (1916) the mortality has been very high—75 deaths out of 95 operations in one series, and 12 deaths out of 15 operations in another. With so high a mortality it may be questioned whether the few survivors would exhibit constant changes referable to pineal deprivation. The mortality is usually due to hemorrhage into the ventricle from injury to the central cerebral veins, or to direct injury to the quadrigeminate bodies or adjacent brain tissues. Dandy has recently developed an operative procedure whereby much of the trauma is obviated. The essential innovation lies in an approach through section of the splenium of the corpus callosum, thus permitting freer manipulations in the operating field. Although the mortality may thus be reduced, the results obtained by Dandy, on comparison with those obtained more recently by Horrax, are uniformly dissimilar. The respective summaries of these two investigators quoted below indicate how incomplete are our available data bearing upon extirpation as a method of approach to the problems of pineal function. Dandy states:

"1. Following the removal of the pineal I have observed no sexual precocity or indolence, no adiposity or emaciation, no somatic or mental precocity or retardation.

"2. Our experiments seem to yield nothing to sustain the view that the pineal gland has any active endocrine function of importance either in the very young or adult dogs.

"3. The pineal is apparently not essential to life and seems to have no influence upon the animal's well being."

These negative findings are in keeping with the earlier work of Exner and Boese, and Biedl. Subsequent to Dandy's publication an extended report has been made by Horrax, whose positive findings are in keeping with those of Foa and Sarteschi. Horrax states:

"1. Total experimental pinealectomy is possible in guinea-pigs and rats.

"2. Pinealectomized male guinea-pigs show a hastened development of the sexual organs, manifested before maturity by a relative increase in size and weight, both of the testes and seminal vesicles, over control pigs of the same litter.

"3. Histologically the testes and seminal vesicles of these animals, if taken before the age of sexual maturity, show a more advanced physiological state than their controls.

"4. The pinealectomized females appear to show a tendency to breed earlier than controls of the same age and weight.

"5. For several reasons, young rats are likely to prove better subjects for experimental pinealectomy than young guinea-pigs, and some evidence of hastened maturity has been obtained in this species."

IMMEDIATE RESULTS FOLLOWING THE INTRAVENOUS OR SUBCUTANEOUS ADMINISTRATION OF PINEAL EXTRACTS.

(Bibliography numbers 58-70.)

Unlike the intense cardio-vascular action of suprarenal extracts, or the uterine contracting action of pituitary extracts, the immediate results from intravenous or hypodermic injections of pineal extracts are not pronounced. Such phenomena as decrease in arterial tension, dilatation of the bloodvessels, altered amplitude and rate of the heart beat, diuresis, glycosuria, and uterine contractions have been reported and confirmed. Under experimental conditions the contraction produced in the uterus by 1 c.c. of 20 per cent pineal extract is much less intense than the contraction produced by 1/200 c.c. of 20 per cent pituitary extract (Fig 2). The intensity of these several activities is so slight that at the present time only technical importance may be attached to these findings.

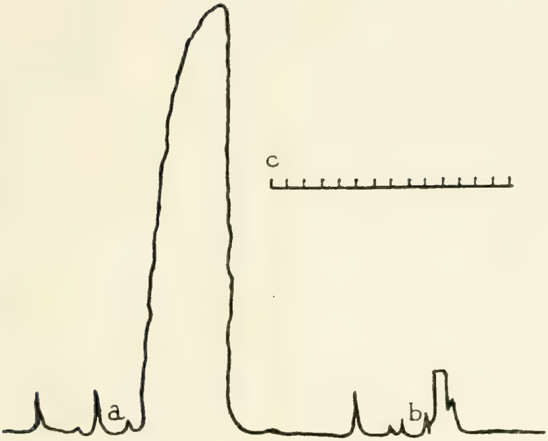

FIG. 2.—A comparison of the effect on surviving guinea-pig uterus of pituitary and pineal-gland extracts. The height of contraction from the pineal extract administered at *B* is trivial in comparison with the contraction at *A* induced by the much smaller quantity of pituitary extract. *C* Time in minutes.

FEEDING EXPERIMENTS WITH PINEAL GLANDS.

(Bibliography numbers 79-87.)

The syndrome of precocious development seen in the human is usually interpreted as the outgrowth of pineal deficiency—a hypopinealism. Such being the case, if the feeding of pineal materials determined any changes, a state just opposite that cited above would be anticipated—a condition of deferred sexual, mental and somatic maturity. Curious to record, feeding experiments lead to rapid sexual and somatic development.

Dana and Berkeley fed pineal materials to young animals (kittens, rabbits, guinea-pigs), and noted a 25 per cent excess in weight over controls. These investigators sought to determine the extent of stimulating influence upon children of low mentality. Fifty feebleminded children were treated and suitably controlled with other children of the same age and diagnosis. Binet tests were the criteria of mental advancement. No physical changes resulted, but on prolonged treatment the mental development was greater than that prior to treatment and in excess of control children of the same mental age. These studies of feebleminded children afford certain technical evidences of value, but the quantity of improvement resulting is not sufficient to warrant any widespread use of pineal materials as a profitable treatment of feeblemindedness.

Hoskins (1916) in feeding experiments upon albino rats, studied the influence upon the growth of the various ductless glands, including thyroid, thymus, hypophysis, and pineal. His results would indicate that none of these glands have any constant effect upon the growth-rate of young rats.

McCord (1914, 1915) employed 400 young animals (chickens, guinea-pigs, dogs) in experiments to establish the extent of influence the pineal exerts upon growth and development. He concludes from his experiments that the same precocity of development usually attributed to pineal deficiency (hypopinealism) was obtained in animals by supplying an increased amount of pineal substance by feeding or injecting pineal preparations. Such administration of pineal substances led to a more rapid growth of body than normal, and determined an early sexual maturity. The excess in rate of growth was most pronounced (40.9 per cent excess in eleven weeks) in young animals fed with pineal tissue obtained from young animals. No tendency to gigantism has followed pineal administration. After maximum size was attained, pineal administration appeared to be ineffective. Both males and females respond to the influence of pineal substances in rate of growth, but the response has been more definitely manifested in males.

II. THE PINEAL GLAND'S INFLUENCE UPON GROWTH AND DIFFERENTIATION.

(*A record of experiments upon postnatal and embryonic growth-differentiation processes.*)

In the developmental processes inaugurated at conception two distinct phases are to be observed—growth and differentiation. In intra-uterine life differentiation into specific organs and tissues is the essential process. In pre-adult life, growth processes are dominant. At puberty differentiation again asserts its influence.

In adult years both these developmental processes are less in evidence, a condition we commonly designate as maturity.

These two phases of development are necessarily intricately interrelated, but within certain limitations may be separately altered. Traces of thyroid tissue added to the water in which tadpoles live will bring about the complete transformation of the tadpole into a miniature frog within one week, whereas normally this metamorphosis consumes from 4 to 6 months (Gudernascht). This phenomenon is due to the intense differentiative action of the thyroid. Similarly thymus tissue retards differentiation of tadpoles. At the period of development wherein normally tadpoles begin to differentiate, thymus fed tadpoles continue to grow larger without differentiation. Manifestly both these factors in development are ultimately dependent upon the quantity or quality of cell activity.

In our earlier records of the influence exerted by the pineal upon development we employed young animals and chicks. Variations were introduced to reduce the possibility of incidental error in dosage, in method of administration, in source of materials, in age of the test animals. With the exception of two series we have uniformly found that young animals which had been fed (or injected) pineal materials have outgrown their controls of the same age. (Fig. 3.) In one series the difference was 40 per cent at 11 weeks of age. No tendency to gigantism was observed. As the normal adult size was approached the stimulative action of the pineal was no longer effective. The testes of certain of these rapidly developed guinea-pigs were examined in comparison with controls. Grossly the testes from pineal fed animals were 50 per cent larger. Microscopically the cellular elements were far in advance of controls and were characterized by very active spermatogenesis. The females gave birth to young when the controls were in the middle third of their gestation. At first it was thought this might be evidence of a shortened gestation period, but more carefully scrutinized experiments determined that this was the outcome of earlier breeding, due to an earlier maturity.

At all times this type of feeding experiment is open to the error that normally such animals exhibit very appreciable individual variations. We have anticipated that less complex life

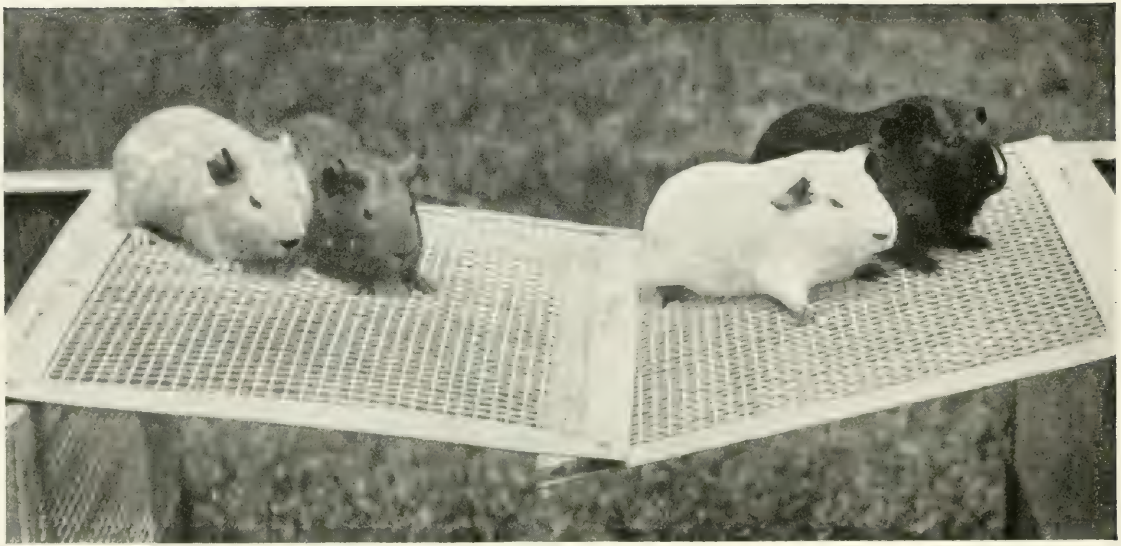

Fig. 3. Results of feeding pineal gland to young guinea-pigs of the same age. Animals to left, controls. Animals to right, pineal fed.

forms that show scant individual variations even in large numbers would afford acceptable data as to any action that pineal extracts might have on their growth-differentiation processes. For the purpose we selected (1) paramœcia (paramœcium caudatum), a unicellular organism that through transverse fission may divide into many generations in a single day, (2) tadpoles of frogs and toads. This larval form of the frog and toad corresponds in many respects to embryonic, intra-uterine life in higher animal life.

PARAMŒCIUM EXPERIMENTS.

Cultures were maintained in the laboratory, growing on hay infusions. These organisms are about ¼ mm. in length and may be readily counted with the naked eye. Through transverse splitting reproduction is accomplished. Under standardized conditions the rate of divisions is relatively constant. It will be argued that in the event of constant exceptional variations in the number of generations formed when pineal materials were added to the culture medium and not occurring when other similar protein materials were introduced, that the phenomenon is attributable to pineal activity. The following procedures were employed. A single paramœcium was isolated until the reproduction of the third generation. These resulting four individuals were separated and placed in different media, (*a*) one in hay infusion, (*b*) one in a hay infusion extract of desiccated pineal gland, .05 per cent strength, (*c*) one in a hay infusion extract of desiccated muscle of equal strength as a control material, (*d*) the fourth one was a variable control. These cultures were maintained in a moist chamber for a fixed period (48 or 24 hours). At the expiration of that time the several cultures were examined as to the numbers of reproductions. Almost invariably the divisions were more numerous in the pineal culture. For example, in one 48-hour experiment 12 individuals resulted from the plain hay infusion, 30 individuals from the hay infusion pineal extract .05 per cent, and 10 individuals from the hay infusion muscle extract .05 per cent. The results from 31 consecutive experiments are grouped in Table 1.

TABLE 4. RECORDS OF DIVISIONS OF PARAMŒCIA.

48-hour group				24-hour group			
No. of Expe'm't.	Hay Infusion.	Pineal Gland.	Muscle Tissue.	No. of Expe'm't.	Hay Infusion.	Pineal Gland.	Muscle Tissue.
1	7	23	..	18	7	13	..
2	7	31	..	19	3	6	..
3	11	12	..	20	11	19	..
4	5	8	..	21	3	5	2
5	1	16	..	22	4	8	8
6	1	13	..	23	6	16	4
7	4	12	..	24	4	8	5
8	5	19	12	25	10	17	10
9	5	12	9	26	11	19	..
10	13	12	4	27	4	7	5
11	2	9	4	28	1	4	3
12	2	7	6	29	1	7	2
13	3	19	16	30	3	8	2
14	1	21	12	31	5	15	5
15	10	25	23				
16	12	30	10				
17	13	22	11				
Average	6.0	17.1	10.7		5.0	10.5	4.6

The inference is, that pineal materials, when added to the culture medium of the unicellular organism, paramœcium, determine a more rapid rate of reproduction. (Fig. 4.)

TADPOLE EXPERIMENTS.

The time of appearance of the successive stages in the differentiation of tadpoles into frogs is an excellent criterion of the influence of any variation in the living conditions of these animals. For the individuals of a single laying, fairly constant is the occurrence of such stages as the budding of the hind legs with the subsequent formation of the different portions of the hind legs, the closing of the gills, the extrusion of the fore legs, the assumption of terrestrial life. Such are the phenomena we sought to influence by the introduction of pineal gland materials into the living water of the tadpoles.

About 50,000 frog eggs were procured and hatched in the laboratory. These were divided into colonies of about 200 each. In most cases these colonies were from the same laying. With so abundant materials it was possible to introduce wide vari-

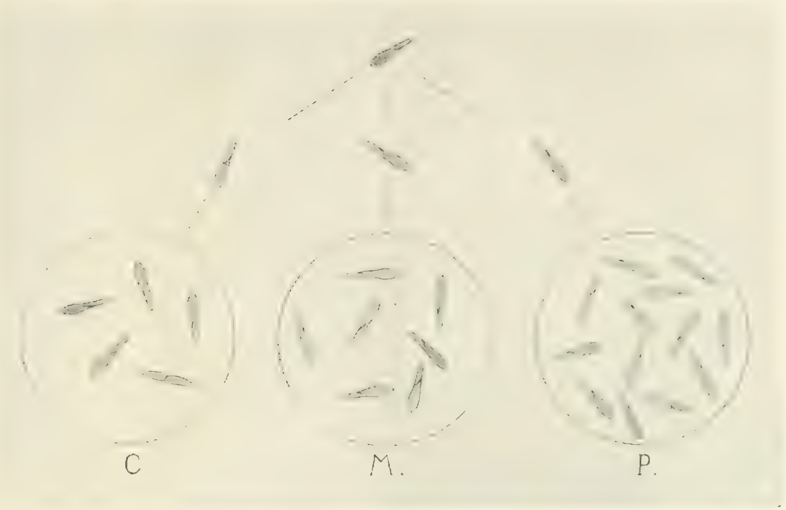

Fig. 4.—A diagram showing the modified division rates of paramœcia (starting with a single paramœcium). *P,* 0.05 per cent. pineal extract in hay infusion. *M,* 0.05 per cent. muscle extract in hay infusion. *C,* hay infusion alone. Diagram represents the average of thirty-six observations.

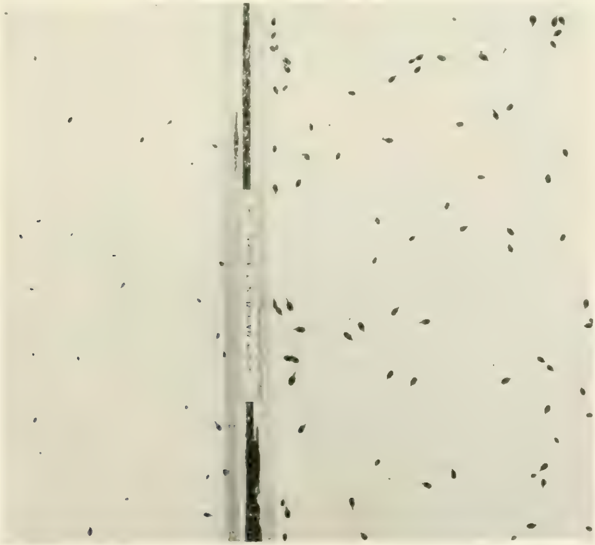

Fig. 5.—A comparison of two groups of toad tadpoles taken from the same lot, photographed simultaneously after two weeks of laboratory feeding. Group to the right fed small amounts of desiccated pineal gland triweekly. Group to the left fed equivalent amounts of desiccated muscle tissue.

ations in test materials and controls. The pineal glands were fractionated into various components and tested against controls such as other endocrine glands, split proteins, histamine, lipoids, etc. Through photography and actual measurements the variations were recorded.

The present paper can make but most casual reference to the accumulated results. The photograph and drawings will serve to indicate the trend of results. (Fig. 5.) (Type photograph—adjacent trays of pineal fed and muscle fed tadpoles of the same laying.) At this stage the pineal fed, while about double the size of the controls, show no tendency to differentiation. Ultimately, however, differentiation is earlier, as may be observed in the drawings of Fig. 6.

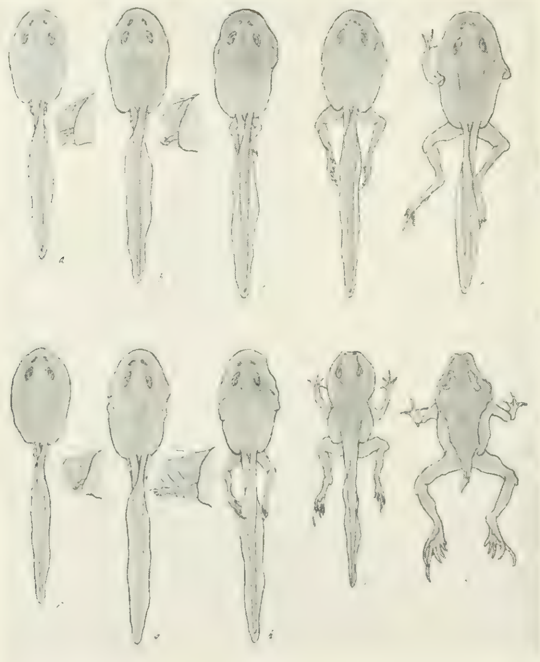

FIG. 6.—Drawings made at weekly intervals indicating the rate of metamorphosis of Bufo Americana tadpoles fed pineal tissue in comparison with normal metamorphosis. *a* to *e* inclusive, controls. *f* to *j*, pineal fed. The small figures to the right of *a*, *b*, *f*, and *g* represent stages in the development of the hind legs for these respective tadpoles.

It is our belief that the pineal gland contains some substance capable of stimulating growth and ultimately differentiation in these larval forms.

GENERAL SUMMARY.

From the lack of unanimity in the literature any conclusions as to the details of pineal gland functions must be made flexible rather than dogmatic. A survey of available data leads to the following summary as representing the present status of the pineal as an organ of internal secretion:

1. A clinical syndrome is to be associated with disturbances of the functions of the pineal gland. Because of the involution of the pineal at puberty, the constitutional manifestations of pineal pathology appear to be confined to prepuberal years. The essential characteristics (apart from pressure and neighborhood manifestations) are (*a*) early sexual development evidenced in the enlarged genitalia, pubic hair, general body hair, early change in voice; (*b*) precocious mental development, manifested in maturity of thought and speech; (*c*) general overgrowth of body to the extent that a child of 6 or 7 years may have the appearance of a child near puberty.

2. The experimental extirpation of the pineal gland is surgically possible. The gland is not essential for the maintenance of life. The early symptoms following pinealectomy are attributable to the severe brain injury. No changes attend the removal of the gland in adult animals. As to the effects of pinealectomy in young animals, Sarteschi, Foa, and Horrax respectively state that the removal of the gland leads to precocity of development. Exner and Boese and Danby report no changes after pinealectomy.

3. The administration of pineal substance to young mammals is reported to hasten growth and sexual maturity. In unicellular organisms (paramœcia) pineal extracts increase the rate of reproduction to more than double that of controls. In larval forms (ranidæ) both growth and differentiation are hastened as a result of pineal feeding.

4. The inference is allowable that the pineal gland is an organ of internal secretion whose functions, however, are of minor significance in the general activities of the endocrinous system.

BIBLIOGRAPHY.

EXPERIMENTAL.

Anatomy and Embryology.

Beraneck, E. Anat. Aneiger, 1892, vii, 674, Anat. Anzeiger, 1893, viii, 669.

Biondi, G. Histologische Beobachtungen an der Zirbeldrüse, Ztschr. f. d. ges. Neurol. u. Psychiat., 1912, ix, 43.

Bizzozero, G. Centralbl. f. d. med. Wissensch., 1871, "R. Istit. Lomb. di Sc. et Lett." Milan, 1871.

Cameron, John. Anat. Anzeiger, 1903, xxiii, Proc. Roy. Soc. Edin., 1904, xxv, 162.

Ramon y Cajal. Apuntes para el studio del bulbo raquideo cerebelo y origen de los nervios encefalieos, Soc. Espana Historia Natural, Madrid, 1895.

Constantini, G. Pathologica, 1910, ii, 439.

Cionini, A. "Riv. Sperim. d. Fren. e. d. Med. Legale," 1885-86, xi, 182; xii, 364.

Cutore, G. Arch. Ital. di Anat. e. di Embryol., Firenze, 1910, ix, 3-4.

Darkschewitsch, L. Neurol. Centralbl., 1886, v, 29.

Dandy, A. Quart. Journ. of Micros. Sci., 1899, xlii, 111. Ibid, 1907, li, 1. Trans. Roy. Soc. Lond., 1911 cci, 227-329.

Dandy, A. The Pineal Gland, Science Progress, 1907, ii, 284.

Dimitrova, Z. Recherches sur la structure de la gland pineale chez quelque mammiferes, Le Nervraxe, 1901, ii, 257.

Edinger, L. Bau der nervösen Zentralorgane, 1908, Ed. 7, 203.

d'Erchia, F. Contributo allo studio della volta del cerebelo intermedio e della regione parafisaria in embrioni di Pesci e di Mammiferi, Monitore zool. ital., 1896, vii, 118 and 201.

Faivre, E. Observations sur le Conarium, Compt. rend. Soc. de biol., 1854, Series 2, i, 195.

Faivre, E. Ann. d. sc. nat., 1857, Series 4, Zoologie, vii-viii, 52.

Fenger, F. The composition and Physiologic Activity of the Pineal Gland, Journ. Am. Med. Ass'n, 1916, lxvii, 1836.

Flesch, M. Anat. Anzeiger, 1888, iii, 173.

Funkqvist, H. Anat. Anzeiger, 1912, xlii, 111.

Galasescu, P., and Urechia, C. J. Les cellules acidophiles de la gland pineale, Compt. rend. Soc. de biol., 1910, lxviii, 623.

Galen. De usu partium Corporis humani, Venice, 1562, viii.

Galeotti, G. Riv. di Patol. Nerv. e. Mentale, 1896-97, ii, 481.

Gaskell, W. H. On the Origin of Vertebrates from a Crustacean-like Ancestor, Quart. Jour. Micr. Sc. London, 1890, xxxi.

Hagemann. "Archiv. von Reichert u. Du Bois-Reymond." 1872.

Hill, C. Jour. of Morphol., 1891, v, 503. Ibid, 1894, ix, 237.

Hoskins, E. R. The Growth of the body of the Albino rat as affected by feeding various ductless glands (thyroid, thymus, hypophysis and pineal), Journ. of Exp. Zoology, 1916, xxi, 295.

Illing, P. Vergl. anat. u. histol. Unters, u. d. Epiphysis, etc. Inaug. Dissert, Leipzig, 1910.

Jordan, H. E. Histogenesis of Pineal Body of Sheep, Am. Jour. Anat. 1911-12, xii, 249.

Jordan, H. E. Results of Recent Studies of the Mammalian Epiphysis Cerebri, Tr. Am. Micr. Soc. 1912, xxxi, 231.

Jordan, H. E. Anatomical Record, 1911, v, 325.

Köllicker, A. Microscopische Anatomie des Menschens, Leipzig, 1850, ii.

Krabbe, K. Sur la gland pineale chez l'homme, (Nouv. iconog. de la Saltpetiere, 1911, Hist. Unters. o. Corp. Pin. Copenhagen, J. Gjellerup, 1915.

Legos. "These de Paris." (A pathological study), 1873.

Leydig, F. "Traite d'Histol. Compt. de l'Homme et des Animeux," 1868, 199.

Locy, W. A. Anat. Anzeiger, 1893, ix, 169, 231, 486. Jour. of Morphol., 1893, ix, 115, 1894; xi 497.

Luys. Systeme Nerveux Cerebro-spinal. 1865.

Meynert, T. Stricker's Handbuch, 1871, Chap. 31.

Münzer, A. Die Zirbeldrüse, Berlin. klin. Wochschr., 1911, xlviii, 1669.

Nicolas, A. Compt. Rend. d. l. Soc. de Biol., 1900, lii, 876.

Obersteiner. Anleitung beim Studium des Baues der nervösen Zentralorgane, 1901, iv.

Peytoureau, S. A. "These de Bordeaux," 1886-87, 68.

Poppi. "L'Ipofisi cerebrale, faringea e la glandula pineale in Patologia," etc., etc., 1911.

Reichert, C. B. Der Bau des menschlichen Gehirns, Leipzig, 1859, Part 2, 69.

Retzius, G. Das Menschenhirn, Stockholm, 1896, 54.

Sarteschi, U. Richerche istologische sulla ghiandola pineale, Folia neurobiol., 1910, iv, 675.

Schwalbe, G. Lehrbuch der Neurologie, Erlangen, 1881.

Seigneur, P. "These de Paris," 1912, 119.

Sorenson, A. D. Journ. of Comparative Neurol., 1894, iv, 153.

Staderini, R. Monit. Zool. Ital., 1897, viii, 241.

Streeter. Mall-Kaibel, 1912, ii, 81.

Studnicka, F. K. Die Parietalorgane, Oppel's Lehrbuch der vergl. mikroskopische Anatomie der Wirbeltiere, 1905, Part 5, Jena. (Monograph).

Soury. "Systeme Nerveux Central.," 1889, ii, 781.

Walter. Beiträge zur Histologie der menschen Zirbeldrüse, Zeitschr. f. d. ges. Neurol. u. Psychiat., Berlin and Leipzig, 1913, xvii, 65.

Warren, J. The Development of the Paraphysis and Pineal Region in Reptilia, Am. Jour. Anat., 1911, ii, 313.

Weigert, C. "Beiträge z. Kenntniss d. normalen mensch. Neuroglia." 1895.

v. Mihalkovicz, V. "Entwick-Geschichte des Gehirns," 1877, 98.

Voeltzkow, A. "Abh. d. Senckenberg, naturf. Gesellsch.," 1903, xxvii.

Biach, P., and Hulles, E. Ueber die Beziehungen der Zirbeldrüse sur Genitale, Wien. klin. Wchnschr., 1912, xxv.

Von Cyon, E. Zur Physiologie der Zirbeldrüse, Arch. f. d. ges. Physiol., 1903, xcviii, 327.

Dixon, W. E., and Halliburton, W. D. The Pineal Body, Quart. Jour. Exper. Physiol., 1909, ii, 283.

Dixon and Halliburton. Proc. Physiol. Soc. (Journ. of Physiol.), 1910, xl, 30.

Howell, W. H. The Physiological Effects of Extracts of the Hypophysis Cerebri and Infundibulæ Body, Jour. Exper. Med., 1898, iii, 245.

Jordan, H. E., and Eyster, J. A. E. The Physiological Action of Extracts of the Pineal Body, Am. Jour. Physiol., 1911, xxix, 115.

Loewy, P. Die Secretwege der Zirbeldrüse, Arb. a. d. neurol. Inst. a. d. Wien. Univ., 1912, xx, 130.

Mackenzie, K. Quart. Journ. of Exper. Physiol., 1911, iv, 317.

Novak, J. Ueber Künstliche Tumoren der Zirbeldrüsengegend, Wien. klin. Wchnschr., 1914, xxvii.

Ott, I., and Scott, J. C. The Action of Corpus Luteum and of the Pineal Body, Month. Cycl. and Med. Bull., 1912, 207.

Ott, I., and Scott, J. C. Proc. Soc. Exper. Biol. and Med., 1910. Therapeutic Gazette, 1911, xxvii, 68. *Ibid.,* 1912, xxviii, 310.

Pellegrine. Gli. effette della castrazione sulla ghiandola pineale, Arch. p. le sc. med., 1914, xxxviii, 121.

Sainton and Dagnan-Bouveret. Descartes et psychophysiologie de la glande pineale, Nouv. iconog. de la Saltpetriere, 1913, xxv, 71-192.

Extirpations.

Adler. Extirpation der Epiphyse, Arch. f. Entwicklungsmechn. d. Organ, 1914, xl, 1.

Exner, A., and Boese, J. Ueber experim. Extirpation der glandula pineale, München. med. Wchnschr., 1911, 154; Neurol. Centralbl., 1910, 754.

Exner, A., and Boese, J. Deutsch. Zeitsch. f. Chirurg., 1910, cvii, 182.

Foa, C. Hypertrophie des testicules et de la crete apres l'extirpation de la gland pineale chez le coq. Arch. ital. de biol., 1912, lvii, 233.

Foa, C. Nouvelles recherches sur la fonction de la gland pineale, Arch. ital. de biol., 1914, lxi, 79.

Sarteschi, U. Richerche istologische sulla ghiandola pineale, Folia neurobiol., 1910, iv, 675.

Sarteschi, U. Sindrome epifisaria macrogenitosmia præcox, Pathologica, 1913, v, 707.

Dandy, W. E. Extirpation of the Pineal Body, Jour. Exper. Med., 1915, xxii, 2.

Horrax, G. Vide infra.

Feeding Experiments.

Berkeley, W. N. The Use of Pineal Gland in the Treatment of Certain Classes of Defective Children, Med. Rec., New York, 1914, lxxxv, 513.

Dana, C. L., and Berkeley, W. N. The Functions of the Pineal Gland, Med. Rec., New York, 1913, lxxxiii, 835.

Goetsch, E. The influence of Pituitary Feeding upon Growth and Sexual Development, Bull. Johns Hopkins Hosp., 1916, xxvii, 29.

Gudernatsch. Feeding Experiments on Tadpoles, Am. Jour. Anat., 1914, xv, 431.

Hofstätter, R. Diskussion zu v. Frankl-Hochwart, Verein f. Psychiat. u. Neurol. in Wien, 1914.

Horrax, G. Studies on the Pineal Gland, I. Experimental Observations, Archives of Internal Medicine, 1916, xvii, 607-626.

McCord, C. P. The Pineal Gland in Relation to Somatic, Sexual and Mental Development, Jour. Am. Med. Assn., 1914, lxiii, 232.

McCord, C. P. The Pineal Gland in Relation to Somatic, Sexual and Mental Development, No. II, Journ. Am. Med. Assn., 1915, lxv, 517.

McCord, C. P. The Pineal Gland, Interstate Med. Jour., 1915, xxii, 354.

GENERAL.

Benda. Deutsch. Klin., 1903.

Biedl. Innere Secretion, Urban and Schwarzerberg, Vienna, 1913.

Cushing, H. The Pituitary Body and Its Disorders, J. B. Lippincott Co., 1912.

Erdheim. Wien. Akad. Sitzsb., 1904, cxiii.

Fröhlich. Wien. klin. Rundschau, 1901, xlvii, 48.

Fischer, B. Hypophysis, Akromegalie und Fettsucht. Wiesbaden, Bergman, 1910.

Kidd, L. J. The Pineal Body. A Review, Rev. Neurol. and Psychiat., 1913, 1.

Münzer, A. Die Zirbeldrüse, Berl. klin. Wchnschr., 1911, xxxvii, 1.

Vincent, S. Internal Secretion and the Ductless Glands, 1912.

Schüller, A. Lewandowsky's Handbuch der Neurologie, 1913, iv, 337.

Marburg, O. Zur Kenntniss der normalen und pathologischen Histologie der Zirbeldrüse, Arb. a. d. neurol. Inst. Obersteiner, Wein, 1909, xvii, 217.

Del Priere, N. Modifications dans la pression sanguine et dans l'accroissement somatique des lapine, a suite d'injections d'extrait de glande pineale, Arch. Ital. de Biol., 1915, lxiii, 122.

CLINICAL.

Askanazy, M. Teratom und Chorionephitheliom der Zirbeldrüse, Verhandl. d. deutsch. path. Gesellsch., Jena, 1906, 58.

Bailey and Jelliffe. Tumors of the Pineal Body, The Archives Int. Med., 1911, viii, 851.

Bartlett, F. K. Hypertrophy of Pineal Gland, The Archives Int. Med., 1913, xii, 201.

Biach and Hulles. Ueber die Beziehungen der Zirbeldrüse zum Genitale, Wien. klin. Wchnschr., 1912.

Blane, G. History of Some Cases of Disease in the Brain: Case of a Tumor Found in the Situation of the Pineal Gland, Tr. Soc. Imp. Med. and Surg. Knowledge, 1800, ii, 198.

Blanquinque. Tumeur de la glande pineale, Gaz. hebd., 1871, 532; Lancet, London, 1871.

Bouchut, M. Kyste du troisieme ventricule avec hydrocephalie des ventricules lateraux, Gaz. d. hop., 1872, xliii, 353.

Campbell, A. W. Notes of Two Cases of Dilatation of the Central Cavity or Ventricle of the Pineal Gland, Tr. Path. Soc. London, 1899, i, 15.

Chirone, P. I'tumori della glandula pineale, Med., ital., 1907, v, 141.

Coats, J. An Adenoid Sarcoma with Cartilage Originating in the Pineal Gland, Tr. Path. Soc. London, 1887, xxxviii, 44.

Cushing, H. The Pituitary Body and Its Disorders, J. B. Lippincott Co., 1912.

Daly. A Case of Tumor of the Pineal Gland, Brain, 1888, x, 234.

Duffin. Tumor Implicating the Corpora Quadrigemina, Clin. Soc. London Lancet, London, 1876, i, 888.

Eberth, J. Intrakranielles Teratom mesodermalen Ursprungs, Virchow's Arch. f. path. Anat., 1898, cliii, 71.

Esteves and Beatti. Klinische und anatomische Studien eines Epithelioms der Zirbeldrüse, Arch. de Pedagogia de la Plata, 1909.

Falkson, R. Ein Chondrocystosarkom im dritten Ventrikel, Virchow's Arch. f. path. Anat., 1879, lxxv, 550.

Feilschenfeld. Ein Fall von Tumor cerebri (Gliosarkom) der Zirbeldrüse, Neurol. Centralbl., 1885, iv, 409.

Förster. Ein Fall von Markschwamm mit ungewöhnlich veilfacher metastaticher Verbreitung, Virchow's Arch. f. path. Anat., 1858, xiii, 271.

Frankl-Hochwart. Ueber Diagnose der Zirbeldrüsentumoren, Deutsch Ztschr. f. Nervenh., 1909, xxxvii, 455.

Frankl-Hochwart. Wien. med. Wochschr., 1910, lx, 505.

Freidreich. Psammoma kystomatosum hämorrhagicum der Glandula pinealis in Kombination mit Medullarsarkom, Virchow's Arch. f. path. Anat., 1865, xxxiii, 165.

Froin. Inflammation meninges avec reactions chromatique, fibrinese et leucocytique du liquide cephalo-rachidien, Gaz. d. hop., 1903.

Garrod, A. E. Pineal Cyst, Tr. Path. Soc. London, 1899, i, 14.

Gauderer, I. Zur Kasuistik der Zirbeldrüsentumoren (Teratoma glandulæ pinealis), Inaug. Dissert, Giessen, 1899.

Goldzeiher, U. Ueber eine Zirbeldrüsengeschwulst, Virchow's Arch. f. path. Anat., 1913, ccxiii, 353.

Gowers. Cases of Cerebral Tumors, Lancet, London, 1879, 394.

Gutzeit, R. Ein Teratom der Zirbeldrüse, Inaug. Dissert, Königsberg, 1896.

Hart, C. Ein Fall von Angiosarkom der Glandula pinealis, Berl. klin. Wchnschr., 1909, xlvi, 2298.

Hedenius, I., and Henschen, F. Tumor in Pineal Gland, Hygeia. Stockholm, 1913, lxxv, 226.

Hempel, K. Ein Beitrag zur Pathologie der Glandula pinealis, Inaug. Dissert, Leipzig, 1901.

Heubner. Demonstration before the Versammlung deutscher Naturforscher und Aerzte, Düseldorf, 1898, Allg. med. Centr. Ztz., 1899, lxviii, 89.

Hijmans van der Bergh and van Hasselt. Tumor glandulæ pinealis, sive epiphysis cerebri, Nederl. Tijdschr. v. Geneesk., 1913, i, 1271.

Horrax. Studies on the pineal gland II Clinical Observation, Archives of Internal Medicine, 1916, xvii, 627-645.

Hösslin, R. V. Tumor der Epiphysis cerebri, München. med. Wchnschr., 1894.

Howell. Tumors of the Pineal Body, Proc. Roy. Soc. Med., 1910, iii, 65.

Hueter. Teratorum der Zirbeldrüse, München. Med. Wchnschr., 1913, 895.

Joukovsky, V. Hydrocephalie et tumeur congenitale de la glande pineale chez un nouveau ne, Rev. mens. d. mal. de l'enf., 1901, xix, 197.

Kny, E. Fall von isoliertem Tumor der Zirbeldrüse, Neurol. Centralbl., 1889, viii, 281.

König, E. Ueber ein Psammosarkom der Zirbeldrüse, Inaug. Dissert, München, 1894.

Lawrence, T. W. P. Tumor of Pineal Body, Tr. Path. Soc. London, 1899, 1.

Lord, J. R. The Pineal Gland; its Normal Structure; Some General Remarks on its Pathology; a Case of Syphilitic Enlargement, Tr. Path. Soc. London, 1899, 1.

Marburg, O. Zur Kenntniss der normalen und pathologischen Histologie der Zirbeldrüse, Arb. a. d. neurol. Inst. Obersteiner, Wien, 1909, xvii, 217; Die Adipositas Cerebralis, Wien. Med. Wchnschr., 1908, lviii, 2617; ibid, 1907, lii.

Marburg, O. Die Klinik der Zirbeldrüse Krankungen, Ergebn. d. inn. Med. u. Kinderh., 1913, x, 146.

Machell, H. T. The Canadian Lancet, 1911, 171. "Report of two cases of sexual precocity."

Massot. Note sur un cas de tumeur cerebrale avec polyurie, Lyon med., 1872, x.

Mestrezat, W. Le liquide cephalo-rachidien, normal et pathologique, Paris, 1912.

Meyer, A. Adenoma of the Pineal Gland, Jour. Nerv. and Ment. Dis., 1903, xxx, 216; *ibid.*, 1905, xxxii, 464.

Morse, J. L. A Case of Abnormal Physical and Sexual Development in an Infant of Two Years. Probably Due to a Tumor of the Pineal Gland, Arch. Pediat., March, 1913, 1.

Müller, E. Ueber die Beeinflussung der Menstruation durch cerebrale Herderkrankungen, Neurol. Centralbl., 1905, 790.

Münzer, A. Pubertas præcox und psychische Entwickelung, Berl. klin. Wchnschr., 1914, x, 1.

Nassetti, F. Contributo allo conocenza dei tumori della gl. pineali, Atti. d. r. Acad. d. fisiocrit di Siena, 1912, v, 19.

Nassetti, F. Pineal cysts, Riv. sper. di freniat, Reggio-Emilia, 1912, xxxviii, 291.

Neumann, M. Zur Kenntniss der Zirbeldrüsengeschwulste, Monatschr. f. Psychiat. u. Neurol., 1901, ix, 337.

Neumann, P. Ein neuer Fall von Teratom der Zirbeldrüse, Inaug. Dissert, Königsberg, 1900.

Niccolai, N. Contributo clinico allo studio delle sindromi epifisarie e funzione endocrine, Riv. crit. di clin. med. Firenze, 1914, xv, 241 and 257.

Nieden. Fall von Tumor (hydrops cysticus) glandulæ pinealis, Centralbl. f. Nervenh. 1879, ii, 169.

Nothnagel. Topische Diagnostik der Gehirnkrankheiten, Berlin, 1879, 206.

Nothnagel. Geschwulst der Vierhügel, Wiener med. Bl., 1888, 162, 193, 225.

Oestreich and Slawyk. Riesenwuchs und Zirbeldrüsengeschwulst, Virchow's Arch. f. path. Anat., 1899, clvii, 475.

Ogle, C. Sarcoma of Pineal Body, Tr. Path. Soc. London, 1899 (two cases).

Pappenheimer. Ueber Geschwülste des Corpus pineale, Virchow's Arch. f. path. Anat., 1910, cc.

Pellizzi, G. B. Riv. Ital. d. Neuropatol, etc., 1910, iii, 193; Lavori d. Ist. d. Clin. d. Mal. Nerv. d. R. Univ. d. Pisa, 1910, ii.

Pineales. Beziehungen der Akromegalie zum Myxödem, Samml. klin. Vortr., New Series, 1899, 242.

Pontoppidan, K. Ein Fall von Tumor der Zirbeldrüse, Neurol. Centralbl., 1885, 553.

Raymond and Claude. Les tumeurs de la gland pineale chez l'enfant, Bull. de l'Acad. de med., 1910.

Reinhold, H. Ein Fall von Tumor der Zirbeldrüse, Inaug. Dissert, Leipzig, 1886.

Rorschach, H. Zur Pathologie und Operabilität der Tumoren der Zirbeldrüse, Beitr. z. klin. Chir., 1913, lxxxviii, 451.

Russel, A. E. Cysts of the Pineal Body, Tr. Path. Soc. London, 1899, i, 15.

Schearer. Enlargement of the Pineal Gland and Sclerosis of Brain in a Case of Chronic Epilepsy with Amentia and Aphasia, Edinburgh Med. Jour., 1875, xxi, 297.

Schmid, G. Ueber latente Hirnherde, Virchow's Arch. f. path. Anat., 1893, cxxxiv, 93.

Schmidt, P. Beiträge Zur Diagnostik der Krankheiten des Gehirns, Med. Ztg., 1837, vi, 32.

Schultz, R. Tumor der Zirbeldrüse, Neurol. Centralbl., 1886, 439.

Segarey, A. Les tumeurs de la glande pineale, Gaz. d. hop., Paris, 1914, lxxxvii, 1141-1205.

Simon, E. Hemorrhagia de la gland pineale, Bull. Soc. Anat., Paris, 1859, xxxiv, 306.

Stanley, S. Dropsy of the Pineal Gland, Lancet, London, 1837, 935.

Thomas. Ueber riesenwuchsähnliche Zustande in Kindesalte, Ztschr. f. Kinderh., 1912, v, 401-430.

Timme, W., and Bailey, P. A Contribution to the Pathogenesis of Progressive Muscular Dystrophy, with the Consideration of Evidence Connecting this Disease with Disturbances in Endocrine Glands. (Abstract) Program 67th Annual Session Am. Med. Assn., 1916, 114.

Turner, F. Ch. Spindle Cell Sarcoma of Pineal Body, Containing Glandular and Carcinomatous Structures, Tr. Path. Soc. London, 1885, xxxvi, 27.

Van der Heide, C. C. Tumor glandular pinealis sive epiphysis cerebri, Nederl. maandscher. v. verlosk. en vronwenz. en v. kindernessk., Leiden, 1914, iii, 253-260.

Verger, M. Gliosarcoma develope an niveau de la glande pineale, Jour. de med. de Bordeaux, 1907, xxxvii, 216.

Verger, M. Teratom und Chorioephitheliom der Zirbeldrüse, Verhandl. d. deutsch. path. Gesellsch., 1906, 58.

Vikhodtseff, S. N. Disease of Pineal Body with Involvement of the Eyes and Acromegaly, Vestnik Ophthalmol., Moskow, 1913, xx, 1014.

Virchow, R. Krankhafte Geschwülste, Berlin, 1863, i.

Weed, L. H., and Cushing, H. Studies on Cerebrospinal Fluid viii, Amer. Jour. of Physiology, 1915, xxxvi.

Weigert, C. Zur Lehre von den Tumoren der Hirnanhänge, Virchow's Arch. f. path. Anat., 1875, lxv, 212.

Wernicke. Lehrbuch der Gehirnkrankheiten, 1883, iii, 299.

Weisenburg, T. H. Brain, 1910, xxxiii, 236.

Wolf. Zirbeldrüsenextrakt in der geburtshilflichen Landpraxis, Deutsch. med. Wchnschr., 1913, xxxix, 1557.

Zenner, A. A Case of Tumor of the Pineal Gland, Alienist and Neurol., 1892, xiii, 470.

Ziegler. Lehrbuch der pathologischen Anatomie, Ed. 4, 620.

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EVIDENCES ASSOCIATING PINEAL GLAND FUNCTION WITH ALTERATIONS IN PIGMENTATION.*

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SEVEN FIGURES.

INTRODUCTION.

In the spring of 1915 we undertook to establish the influence of pineal gland substances upon growth and differentiation processes in tadpoles. The tadpoles employed were hatched in the laboratory and immediately placed upon a diet of pineal gland. On the tenth day of larval life it was readily observable that in the pineal fed groups the coloration was uniformly lighter than in the control, muscle-fed groups. This alteration was at first attributed to some unknown difference in environmental conditions—light, background, etc., as the color of these organisms was known to vary considerably in response to such stimuli. These changes when first noticed were trivial in degree, but as development progressed the alterations in pigmentation became correspondingly greater. Thirty minutes after feeding pineal tissue, the tadpoles which prior to the feeding had been uniformly dark, became so translucent that all the larger viscera were plainly visible through the dorsal body wall (figs. 1 and 2). This translucency appeared with such regularity and so punctually after pineal feedings and was so markedly absent in the control groups that the phenomenon was made the subject of special study. Out from this work have come many acceptable evidences of a pineal gland influence upon pigmentation, upon the phases of colloidal state, and upon the vegetative nervous system.

The following report is the record of the unfolding of this further work.

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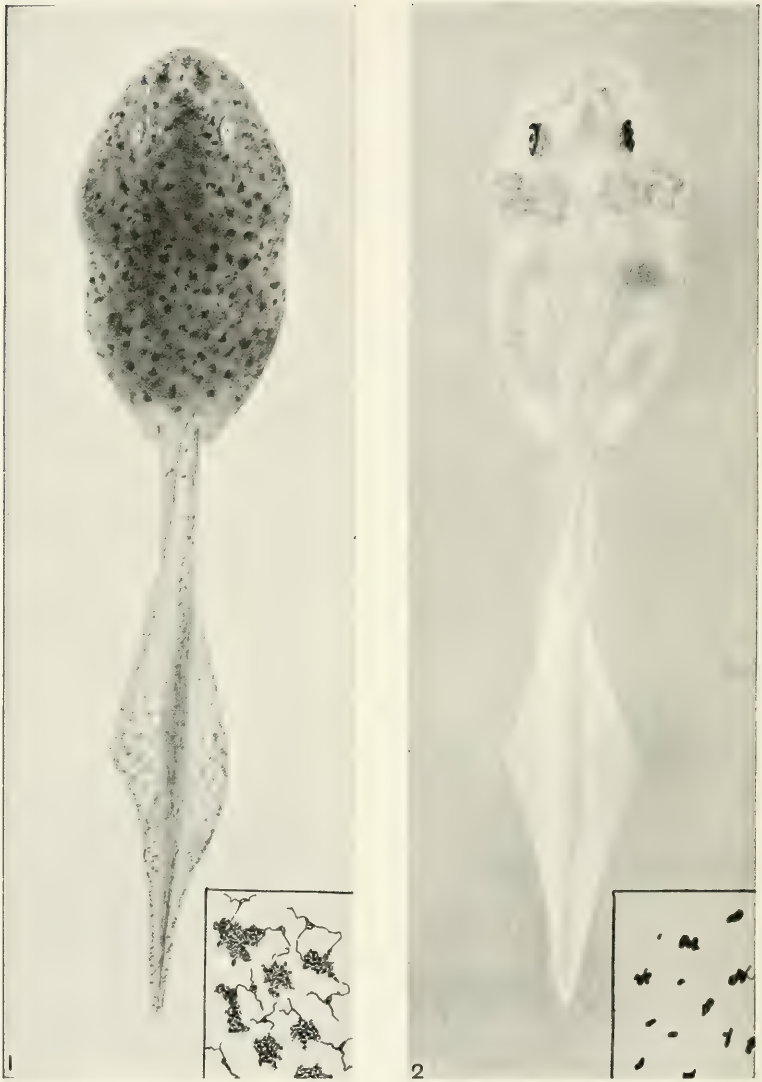


FIGS. 1 and 2. Drawing of the same tadpole just prior to (Fig. 1) and forty-five minutes after (Fig. 2) pineal feeding.

NATURE OF PIGMENTATION.

The color phenomena observable in many animal life forms are due to the absorption or reflection of light rays by chemical substances in the integument of the animal. These materials are usually found as granules of pigment lying in specialized cells, the chromatophores. Chromatophores are divisible into several types, but of these only two are found in frog skin. First, the melanophores, lying in the skin, peritoneum, etc., contain granules of dark-brown or black melanin and are often contractile. Second, the xanthophores, which are found only in the adult skin, contain granules of light yellow xanthin and are never contractile.

The relationships between pigmentation and environment are perfectly obvious but little understood. The color changes of the common tree toad (Hyla arborea) have been accounted for in various ways. One writer holds that the pale condition is the result of the stimulative effect of light upon the chromatophores and that the dark phase is due to the absence of that stimulant (1). Another observer (2) claims that light alone has very little effect, but that changes of temperature control the coloration. The problem is complicated by the fact that the same individual may not react in the same way under exactly similar conditions. Certain it is, however, that animals do respond to environmental changes by alterations in their coloration. It has been shown by numerous experimenters that these changes are to a great extent under the control of the eyes. A change of environmental light is caught by the eye and the resulting stimulus transmitted to the chromatophores by the central nervous system. That this is the usual method of procedure is indicated by the atypical reactions of blinded individuals.

The mechanism of these changes is entirely dependent upon the contraction or expansion of the melanophores. Both xanthophores and melanophores enter into the color effects, but the former play a passive role and always present the same appearance. The melanophores of frog tadpoles are of two distinct types. The simpler and less conspicuous form is limited to the epidermis. It consists of a cell-body with two or more simple processes (*Ep. M.*, figs. 5 and 6). They may lie singly in the epidermis or in such abundance as to form a definite reticulum. These melanophores are not contractile (3).

The second type of melanophore is found in greatest abundance in the subepidermal connective tissue. During late metamorphosis these cells migrate to the corium. In the expanded condition the subepidermal melanophores present a very typical "mossy" appearance. The cells lie so closely approximated that they form a nearly continuous sheet (*Sub. M.*, fig. 6). There is a lighter, central space in each cell probably representing the nucleus. In the contracted condition (*Sub. M.*, fig. 7) the melanophores appear as irregular dots in which no structure is visible.

It has been fairly well established that the contractile melanophores are innervated by motor fibres proceeding along both sympathetic and spinal nerve paths (4). Hooker has shown physiologically that the reactions of the melanophores of the frog are synchronized by the action of the central nervous system. By histological methods, Ballowitz (5) has demonstrated motor nerve endings in melanophores of bony fishes. Laurens (6) working with Amblystoma larvæ has shown that the melanophores may contract as the result of direct stimulation. Often, however, this primary reaction is overcome by an opposite, secondary reaction initiated by the central nervous system.

There are three principal theories to account for the mechanics of melanophore contraction. Ballowitz (1) claims that the contraction is an intra-cellular migration of the pigment granules within fixed cells. The protoplasm of a chromatophore is filled with numerous, extremely fine, radially arranged, anastomosing canals within which the pigment is forced back and forth by the alternate contraction and relaxation of the protoplasmic canal walls.

Spaeth (7) believes that the chromatophores of fishes are fixed stellate cells, within which the pigment granules, carried in a rather fluid cytoplasm, stream into and out of the processes during expansion and contraction. He explains this migration as a strictly colloidal phenomenon, the contracted and expanded conditions representing respectively the aggregate and disperse phases of a colloidal suspension. This is perhaps the most widely accepted and tenable view.

Hooker (8) has advanced a third explanation. He believes that the melanophores of frog larvæ lie in preformed spaces and that the cells expand and contract as a whole within the spaces which enclose them. The acts of expansion and contraction, according to this theory, are brought about by pseudopodia, the pigment granules being carried in the cell cytoplasm. On this premise the pigment cells are to be considered amœboid.

These expansions and contractions are commonly brought about by changes in the intensity of light or heat, but many other agencies will cause a specific reaction. Spaeth (7) has made a detailed study of the reactions produced by a great variety of stimuli upon the melanophores of Fundulus. He notes that the reactions are in every way comparable to those obtained by the same agents upon smooth muscle. He raises the very pertinent question as to whether melanophores may not be considered as modified smooth muscle cells.

In 1910 Babak (9) noted the reversal of the normal reaction to light when Axolotl larvæ were blinded. In diffuse light the melanophores of normal seeing larvæ contract. After painting the eyes with an opaque substance the melanophores expand in light. In the same way the melanophores of normal larvæ expand in darkness, while those of blinded larvæ contract. Fuchs (10) explained the phenomenon as due to the intervention of the parietal organ (the pineal gland of higher organisms). He reached this conclusion from a consideration of the phylogeny of this organ. The embryology of the parietal organ in some of the lower reptiles indicates very clearly that this body is a remnant of a third eye. Fuchs assumed that it had retained some of its controlling power over the melanophores. In the normal larvæ its influence is completely overshadowed by the superior power of the functioning eyes. In the blinded larvæ the parietal organ again assumes control. Laurens has completely disproven this hypothesis (11).

It is noteworthy from the present study that although the pineal gland does not exert a controlling influence upon pigmentation comparable to that arising from environmental stimulation of the retina, nevertheless it contains an active substance capable of directly inducing pigmentation changes irrespective of environmental conditions.

MATERIAL AND METHODS.

Eggs of the species Rana pipiens, Rana cantabrigiensis and Bufo Americana were collected in the vicinity of Detroit, and hatched in the laboratory. Immediately after hatching and before the oral orifice had opened they were grouped in trays into colonies of 200 and food placed in the trays. The food was weighed, each colony receiving the same amount triweekly. All foods were taken voraciously by the tadpoles. By means of a water-dropping and disposal system the tadpoles were at all times in fresh, aerated tap water. Moreover the trays were frequently shifted to average environmental conditions of light and temperature. In the observations on pigmentation we used some 12,000 tadpoles.

The food consisted of desiccated glandular material and fresh plant food. Of the glands we tested the effect of pineal (adult and preadult) thyroid, parathyroid, and suprarenal. Brain tissue and beef muscle were used as controls. Different lots of tadpoles were fed upon Spirogyra, bread crumbs and hemp seed as a further check. A single lot of tadpoles was fed on desiccated retinæ from beef eyes as a particular experiment. Of these tissues the pineal gland alone produced the phenomenon we have called the pineal-pigment cycle.

EXPERIMENTAL EVIDENCE.

Effect of whole pineal tissue on pigmentation.

Certain endocrinous tissues are known to alter pigmentation in tadpoles. This was noted by Gudernatsch (12), in his feeding experiments on these animals. Concerning the pigment altering tissues he states:

> "After two weeks feeding, those (Tadpoles) fed on adrenal cortex became much lighter than those fed on adrenal medulla or any other food. This difference in color became more evident as the experiment proceeded, until the cortex-fed tadpoles had an extremely light, greenish yellow tint. The spleen and thymus fed tadpoles became extremely dark during the course of the

feedings. Those fed on liver developed a dark greenish color, those on ovary a yellowish color."

The type of pigment variation observed by Gudernatsch is obviously distinct from that observed by us. In his animals the pigment alteration was slow in appearing and persistent. In ours the change appeared early in life, occurred sharply in relation to feeding, was cyclic and transient.

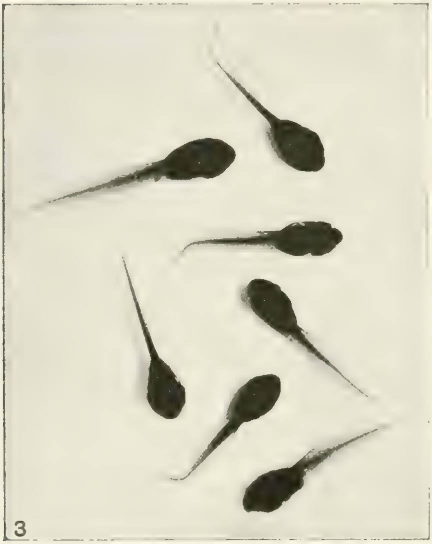
After the tenth day of larval life pigment changes were always evident after every feeding of the pineal tissues, and the animals continued to react until their forelegs protruded. Sufficient blanching of the bodies occurred within thirty minutes after pineal feedings to differentiate these colonies from their controls. A maximum condition of translucency was attained in about forty-five minutes, and three to six hours later restoration to the original color was complete. The difference was first noticeable in the region about the eyes due to the absence of larger viscera. It can be demonstrated, however, that the reaction occurs simultaneously over the whole body. At the height of the reaction the integument was so transparent that the brain, the olfactory tracts, the kidneys, the beating heart, and the intestines were all clearly visible through the dorsal body wall.

Figures 1 and 2 are drawings of a single tadpole just prior to and forty-five minutes after feeding pineal material. The darker portions in 2 are due to the denser viscera, the pigment conditions being the same over the entire animal.

Photography fails to give a true picture of this phenomenon but since actual photographs are more valuable as exact evidence than drawings figures 3 and 4 are here included. These are respectively photographs of the same group of tadpoles just prior to and thirty minutes after feeding 5 mgm. fresh pineal gland. A true evaluation of the relative pigmentation may be had by comparison of the tadpoles of figure 3 with the periphery of those in figure 4. The dark color in the center of the bodies in figure 4 is due to the opacity of the denser viscera and intestinal contents and not to a difference in the pigmentation of the skin.

If a portion of the skin from a light and from a dark tadpole be dissected loose and examined under a microscope the reason for the difference in shade will be readily apparent (figs. 6 and

7). The two types of melanophores are present. In the normal (dark) piece of skin the subepidermal melanophores (fig. 6, *Sub. M.*) are expanded to such an extent that they form an opaque sheet in which there are left a few scattered openings. In the light piece of skin these melanophores are contracted to rough spheres of pigment (fig. 7, *Sub. M.*). The epidermal melanophores exhibit an unchanged appearance in both drawings. A sagittal section of normal skin (fig. 5) shows the relation between the two types of melanophores and the various layers of the integument.



FIGS. 3-4. Photographs made by reflected light.

FIG. 3. Normal tadpoles—just prior to pineal feeding.

FIG. 4. Same tadpoles 30 minutes after feeding acetone extract of pineal gland. The darker portions of these tadpoles are due to denser viscera—heart, gills, intestinal contents, etc. The degree of translucency is identical in all parts of the skin.

These described alterations in pigmentation are invariably induced in tadpoles upon the administration of pineal materials, be they the fresh minced glands, simple desiccation preparations, or simple aqueous extracts. In an effort to associate these changes with certain constituents of the pineal gland, various fractions of the pineal were prepared and employed and are now about to be described.

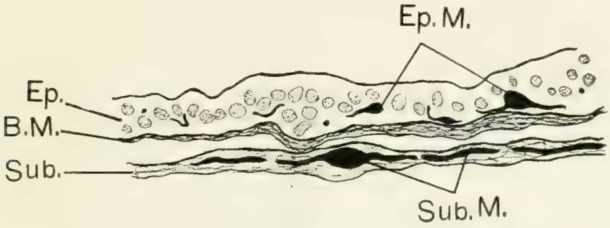


FIG. 5. Sagittal section of integument taken from the eye region of a normal tadpole. Section illustrates the two types of melanophores and their position relative to the tissue layers.

ABBREVIATIONS.

Ep., epidermis	*Ep.M.*, epidermal melanophores
Int., integument, including epidermis and subepidermal tissue	*Sub.M.*, subepidermal melanophores
	B.M., basement membrane
	Sub., subepidermal connective tissue

Effect of pineal fractions on pigmentation.

In the preparation of these split materials the fresh glands were either ground up and immediately extracted or desiccated and subsequently extracted. From the results of a wide variation in fractionation methods, chief interest centers around the acetone and alcohol extractives and their residues. In the case of acetone the process was carried out in a Soxhlet apparatus. On freeing the extractives from acetone there resulted a brownish-black fatty mass with an odor suggestive of crude fish oil. Portions of these extractives and the residue were preserved intact for experimentation. Other portions of both were re-extracted with alcohol. Likewise fresh pineal material was extracted with alcohol and the residue and extractives respectively extracted subsequently with acetone. These several preparations were tested as to their influence upon pigmentation on several hundred tadpoles from the same hatchings. At once it was apparent that the pigment altering principle was completely dissolved in acetone. The typical pigment cycle was induced by this extract while the residue and all acetone extracts of muscle

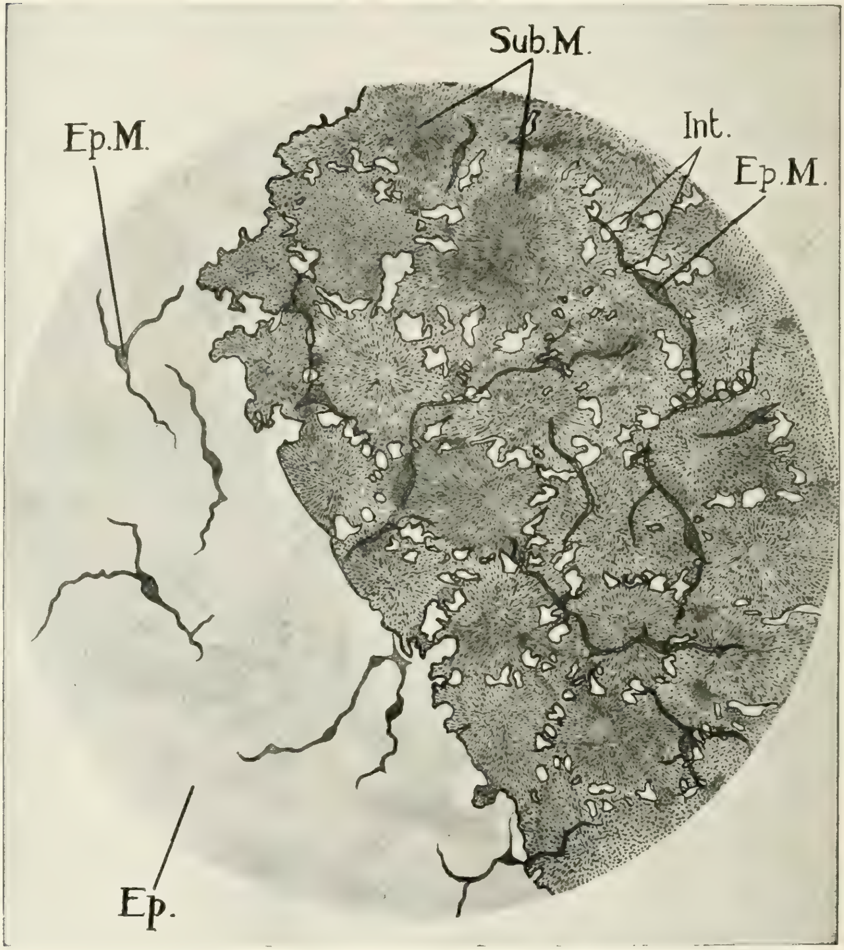


FIG. 6. Surface view of integument from normal tadpole. On the left side of the drawing the subepidermal connective tissue has been torn away, leaving the epidermis alone. Thus on the right side both types of melanophores are visible, on the left the epidermal type alone. The pigment granules in the subepidermal melanophores are evenly distributed throughout the cytoplasm, illustrating the disperse phase.

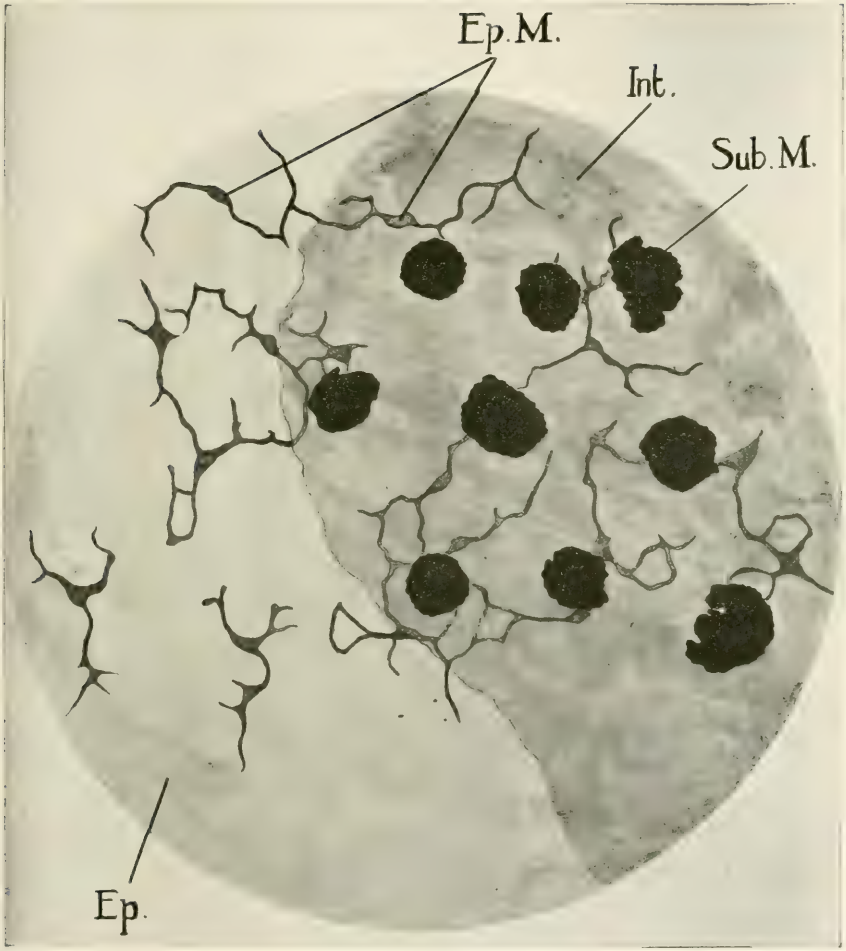


FIG. 7. Similar view of integument from tadpole fixed 30 minutes after feeding acetone extract of pineal gland. In this case the pigment granules in the subepidermal melanophores are collected into a mass in the center of the cell—the aggregate phase.

The material was fixed in Bouin's fluid, dehydrated, cleared in xylol and mounted without staining in balsam. The drawings were made with the aid of an Abbe camera lucida, with Leitz objective 6 and ocular 4, giving an approximate magnification of 590 diameters, but have been reduced ⅓ for publication. All material was taken from the region between the eyes.

tissues induced no pigment changes. The residue from acetone extraction was, however, capable of inducing the growth stimulating action that McCord has described for the pineal gland, while the acetone extracts which were exquisitely active in inducing pigment alterations were only slightly active in stimulating rapid growth. The inference is that at least two separate distinct principles exist in the pineal, the one producing the pigment phenomena, the other stimulating rapid growth. In the case of the alcoholic extraction the active substances were not readily soluble, for the alcoholic extractives, the alcoholic residue, and the acetone reëxtractives, all yielded positive pigment results. The acetone extractives yielded quite readily to aqueous emulsifying, and this form proved to be the most convenient mode of employing this material.

Quantitative relations in time and amount of contraction.

In the feeding of pineal materials to the tadpoles the time interval necessary to establish maximum contraction of the pigment cells increased as the concentration decreased. Tadpoles placed in a 1:500 pineal emulsion were noticeably lighter in five minutes and required but thirty minutes to arrive at maximum translucency. In higher dilutions the maximum translucency was attained only after a longer interval, and in very high dilutions producing only qualitative changes the maximum was not attained. The dilution of 1:100,000 was the highest that produced a macroscopically discernible qualitative action. These reasonably constant quantitative relations have afforded us a means for the evaluing of the strength of our several preparations and may on extended study prove to be a trustworthy method for the standardization of pineal products, for at the present time no method exists for testing the strength of such preparations.

The quantitative relations between concentration and time will be evident from the following table:

Dilution of Acetone Extract	Maximum Reaction Attained in
	minutes
1 : 500	30
1 : 1000	45
1 : 2000	60
1 : 5000	105
1 : 10,000	Qualitative change but maximum not attained.
1 :100,000	Maximum not attained.

The maximum reaction was determined by comparison with a standard consisting of several tadpoles which had been placed in a 1:500 pineal emulsion thirty minutes prior to the beginning of the experiment. The translucency thus obtained was found to be the greatest possible. It served as the criterion for comparison as to the degree of depigmentation induced by pineal preparations of unknown activity. In the practical standardization of pineal preparations, the end reaction of greatest feasibility was the comparative time intervals necessary to attain to maximum translucency. Such a method is obviously open to the criticism that frog larvæ are only obtainable for a short period in the year. With Bufo Americana pigment changes were found to be too trivial to be of value in standardization. Rana pipiens were exquisitely responsive and admirably suited for standardization purposes except in that only during the spring months are they obtainable. We are now experimenting with certain amphibian larval forms that may be obtained throughout the year.

We have determined that the growth stimulating principle in the pineal is distinct from the principle concerned in pigment changes, and this on further investigation may militate against this proposed means of standardization.

Effect of pineal gland upon melanophores of other forms.[1]

Pineal gland extracts have no demonstrable effect on either type of chromatophore of Loligo (squid) or upon the melanophores of adult Fundulus. The extracts however determine distinct contraction of the melanophores of young Fundulus as may be noted in accompanying table.

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[1] Experiment conducted by A. Noble at Woods Hole, Mass.

No. 1 One-Week-Old Funduli in Boiled Sea Water.		No. 2 One-Week-Old Funduli in Emulsion of Acetone Extract of Beef Muscle in Sea-Water.		No. 3 One-Week-Old Funduli in Emulsion of Acetone Extract of Pineal Glands in Sea-Water.	
Time		*Time*		*Time*	
9.15	Complete expansion of melanophores	9.15	Complete expansion.	9.15	Complete expansion
9.38	Complete expansion of melanophores	9.40	Complete expansion.	9.37	Beginning contraction
9.46	Complete expansion of melanophores	9.47	Complete expansion.	9.45	Contraction increasing
9.59	Complete expansion of melanophores	10.00	Complete expansion.	9.58	Contraction almost complete
10.31	Complete expansion of melanophores	10.32	Complete expansion.	10.30	Complete contraction
11.51	Complete expansion of melanophores	11.52	Complete expansion.	11.50	Complete contraction
11.55	All transferred to fresh sea water				
1.55	p.m. Complete expansion	1.56	Complete expansion	1.54	Partial expansion
3.01	p.m. Complete expansion	3.02	Complete expansion	3.00	Complete expansion of some of the melanophores, especially in tail; partial expansion of others

Mode of absorption of the pigmentation altering principle.

Several experiments were tried with the object of showing whether the pineal tissue must be ingested or not in order to produce the reaction.

1. *Pineal emulsion on anesthetized tadpoles.* Several tadpoles of equal depth of pigmentation were completely anesthetized with ether, after which half were placed in an emulsion of acetone extract of beef pineal, the others in a like emulsion of

muscle tissue. Five minutes later those in the pineal emulsion were perceptibly lighter, later acquiring a marked translucency. The latter remained unchanged. The tadpoles recovered from the anesthetic and gradually regained their original appearance.

2. *Hypodermatic injection of pineal emulsion.* Several tadpoles of equal depth of pigmentation were divided into two groups. One received 0.01 cc. of an emulsion (1-500) of acetone extract of pineal gland, injected hypodermatically into the peritoneal cavity; the other received the same amount of normal saline solution injected in a similar way. Shortly after injection the pineal treated animals became lighter, eventually reacting to the maximum degree of translucency. The other tadpoles remained practically unchanged in appearance. As a further control a third group was immersed in the pineal emulsion, for the length of time consumed in making the injection and the tadpoles were then washed in tap water. They remained unchanged. This control showed that the effect produced was due to the injected pineal material and not to any accidental absorption through the skin.

3. *Effect of pineal emulsion on eviscerated tadpoles.* A number of tadpoles were completely eviscerated. These tadpoles live and swim about as freely as their fellows. Following this procedure part of the animals were removed to an emulsion of the acetone extractive of beef pineal gland and shortly passed through the same pigment changes as normal tadpoles placed in this emulsion at the same time. The eviscerated and normal tadpoles remaining in tap water did not change in color.

These observations prove conclusively that the principle involved is directly absorbable through the gills or skin. The effect is produced without the intervention of the processes of digestion. There is no indication, however, as to whether the principle acts directly upon the melanophores, or indirectly through the medium of the central nervous system.

Effect of pineal extract upon unstriated muscle.

Aqueous extracts of fresh pineal glands were tested according to the method of Dale and Laidlaw (13) upon isolated strips of guinea-pig uterus. The extract produced a typical though feeble contraction of the uterine muscle. Three cc. of a 20-per-cent pineal extract was roughly equivalent in activity to 0.004 cc. of a 20-per-cent pituitary extract. Thus pineal extracts stimulate certain smooth muscle cells as well as pigment cells to contraction. This similarity of action goes far to confirm Spaeth's hypothesis that the melanophore is a type of smooth muscle cell.

COMMENT.

Many acceptable evidences associate the pineal gland with an earlier optical function. In the reptilian stage of evolution this parietal eye probably attained to its highest development. In the embryos of certain lizards (Lacerta agilis) the typical eye structure is still evident, but in no form living at the present time does the pineal gland retain an ocular function of high order (14).

The color changes in certain forms are obviously in adjustment to environmental conditions. The eye is the essential controlling factor in this adjustment. When in blinded animals certain definite changes in pigmentation still occur, on theoretical ground it is tenable to assume that the pineal body retains sufficient ocular mechanism to exert its influence upon the pigment cells. This is the hypothesis suggested by Fuchs (10). Laurens has established experimentally that such an activity on the part of the pineal is highly improbable (11). Accepting the contentions of Laurens, it is the gist of our work that while the pineal does not act in the rôle of its ancient ocular function, it contains within itself an active principle capable of inducing pigment changes independent of and wholly apart from environmental conditions. The pineal pigment changes dominate and appear despite environmental conditions tending toward the opposite phase. The salient observations that we have recorded in detail on the foregoing pages are:

1. Up to near tenth day of larval life in tadpoles, pigmentation is not influenced by pineal feeding. Evidences relative to this are not precise, but suggest that this is due to incomplete development of the nervous mechanism involved.

2. Beginning at this time and continuing until near the termination of metamorphosis, the addition of traces as small as 1 part acetone extract in 100,000 parts water determine distinct cyclic pigment changes peculiar to these preparations. Prior to

feeding, both controls and experimental animals are uniformly dark colored. Shortly after feeding the pineal fed groups begin to lose color until within thirty minutes all macroscopic pigment is lost so that all the larger viscera are clearly visible (figs. 1 and 2). The condition is transient and the cycle is complete with full restoration of color within from three to six hours, unless further pineal food is added to the trays. As metamorphosis is completed the pigment is no longer altered by pineal materials, due to rearrangements of chromatophore types and sites in the adult animal.

3. The response in pigment change is quantitative. A method is described for the standardization of pineal preparations.

4. The pineal substance responsible for the pigment changes is wholly extracted by acetone. The residue after acetone extraction is an inert substance as to pigment influence. However this residue has an influence on growth and differentiation. The inference is that the gland contains more than one active substance.

5. The reactions produced by pineal extracts add some evidence to Spaeth's contention that the melanophores are modified smooth muscle cells. The similarity of contraction of certain smooth muscle organs under the influence of pineal extracts and the contraction of melanophores is in keeping with Spaeth's hypothesis.

The very nature of this pineal-pigment cycle affords an excellent method of approach to the mechanics of melanophore function and from this the larger problems of colloidal state.

LITERATURE CITED.

(1) Ballowitz, E. 1914. Pflüger's Archiv, Bd. 157, 165.
(2) Hargitt, C. W. 1912. Jour. Animal Behav., vol. 2, 51.
(3) Hooker, Davenport. 1914. Science, N. S., vol. 39, 473.
(4) Hooker, D. 1912. Zeit. f. allg. Physiol., Bd. 14, 93.
(5) Ballowitz, E. 1893. Zeit. wiss. Zoöl., Bd. 56, 673.
(6) Laurens, Henry. 1915. Jour. Exp. Zoöl., vol. 18, 577.
(7) Spaeth, R. A. 1916. Jour. Exp. Zoöl., vol. 20, 193.
(8) Hooker, D. 1914. Am. Jour. Anat., vol. 16, 237.
(9) Babak, E. 1910. Pflüger's Archiv, Bd. 131, 87.
(10) Fuchs, R. F. 1915. Winterstein's Handb. d. vergl. Physiol., Bd. 3, Teil 2, 1189.
(11) Laurens, H. 1916. Jour. Exp. Zoöl., vol. 20, 237.
(12) Gudernatsch. 1914. Am. Jour. Anat., vol. 15, 431.
(13) Dale and Laidlaw. 1912. Jour. Pharm. and Exp. Ther., vol. 4, 75.
(14) Piersol, Human Anatomy, 4th ed., vol. 2, 1124.

103806

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DESICCATED ANTHRAX ANTIGEN FOR IMMUNIZATION PURPOSES.

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Pasteur's anthrax vaccine has been used, wherever anthrax has prevailed, since it was first brought to the attention of the veterinary profession, although it has long been known to be an exceedingly unstable and unreliable product. According to Washburn (Farmer's Bulletin No. 784, United States Department of Agriculture), "In the Pasteur method of vaccination there are, however, disadvantages which must be duly considered. To obtain satisfactory results from the use of Pasteur's vaccine it is of primary importance that the product be active. Experience has proved that this type of vaccine, if subjected to unfavorable conditions, may deteriorate within a short time after its preparation. Since the enactment of legislation giving the United States Department of Agriculture control of the manufacture of veterinary biological products going into interstate trade, periodical tests have been conducted with anthrax vaccine prepared by various manufacturers, and in many instances the vaccine proved inert within three months of its preparation. In other cases it remained potent for a year. When exposed to warm temperature and light it deteriorates very rapidly, and when it is remembered that the products of manufacturers may be stored under unfavorable conditions in branch houses and in rural drug stores, the loss of value can be readily explained."

It seems to be requisite, for the successful vaccination of animals against anthrax, to artificially produce a slight attack of the disease. This procedure raises the resistance of the tissues of the animal sufficiently to prevent the live virulent microörganisms from finding lodgment and thus producing the disease in the natural way. The organisms in Pasteur's vaccine do not remain in a sufficiently virulent condition, as a rule, to produce this artificial immunity, so that investigators, realizing this fact, have endeavored to devise other methods of preparing the antigen for vaccine purposes. The very fact that the anthrax bacillus is a spore-bearing organism very readily lends itself to the solution of the problem, as it is known that the spores at times retain their virulence for years under circumstances that would prove detrimental to organisms in the non-spore or vegetative stage, such as Pasteur's vaccine. Experiments have shown also that when the anthrax organism is acted upon by the proper amount of heat it gradually loses its virulence and, thus attenuated, will retain its modified strength in the spore stage. The method that is employed for this attenuation is that first proposed by Pasteur and used for his vaccine. A broth culture of the anthrax bacillus is allowed to grow under the influence of a temperature of 42.5° C. and, at varying intervals, is tested for virulence on rabbits, guinea pigs and white mice. A suspension of the organism just virulent enough to kill a white mouse but not a guinea-pig is designated as vaccine No. 1, and one that will kill a guinea-pig but not a rabbit is vaccine No. 2. With this method of procedure it is a very simple matter to standardize a spore vaccine to any strength.

According to Eichhorn (Bulletin No. 340, United States Department of Agriculture), Zenkowsky in Russia, Detre in Hungary, Nitta in Japan, and others, have had successful results with spore vaccines, and for that reason and also as a result of experiments carried on by the U. S. Bureau of Animal Industry, a spore vaccine has been proposed by them to replace, in this country, the Pasteur vaccine, using for the spore vaccine the same standardization tests as for Pasteur's vaccine. In the preparation of this spore vaccine the following method is given by Eichhorn: "For the purpose of producing a spore vaccine it is desirable to use a peptone-free agar media and after inoculation with the attenuated culture to grow the organism at a temperature of 37.5° C. for 4 to 7 days, by which time an abundance of spores will have formed. The growth is then washed from the slants and collected in a sterile flask and heated to a temperature of 60° C. for one-half hour, to destroy the vegetation forms of the organism. A measured quantity of this suspension can then be plated out in the usual manner and the spore control of 1 c.c. of the suspension be established." He also says, "In consideration of the keeping qualities of the spore vaccine, large lots can be prepared without fear of deterioration. In the bottling and storing of the same, however, proper care should be taken to prevent contamination."

In preparing material of an antigenic nature, especially for vaccine purposes, experience has taught us that deterioration due to light, heat, chemical action, autolysis, bacterial contamination and other conditions, is a factor with which one must constantly contend, and a factor best controlled by refrigeration. For therapeutic immunization on a practical basis, however, refrigeration is out of the question, and desiccation has of late proven a satisfactory substitute.

While the spore vaccine in suspension used in Russia, Hungary and Japan and advised in this country by Eichhorn and others has a distinct advantage over Pasteur's vaccine, it also has its faults, and it is very evident that the ideal vaccine would be a spore vaccine in a desiccated form. This sort of a vaccine would embody all of the advantages of both the spore vaccine and Pasteur's vaccine. With this thought in mind experiments were undertaken by the author to determine, if possible, the practicability of a vaccine.

The desiccated spore vaccine in question was prepared in a similar manner to the spore vaccine in suspension, with the exception that the growth of the anthrax bacillus was scraped off the agar, incorporated with a sterile diluent in a proportion suitable to fulfill the tests required for its standardization, and dried at room temperature.

This vaccine was first tested in the early part of last year, and upon repeating the tests one year later, with the same vaccine which had, in the meantime, been kept at room temperature, it was found that there was no deterioration in its virulence. This showed conclusively that it is not even necessary to keep the desiccated vaccine at refrigerator temperature as is advised for the ordinary spore vaccine in suspension.

There is also a question of vital importance to owners of cattle, especially when the animals are raised in large numbers, relative to the form in which the desiccated material is presented and the way in which it is to be administered. This is an extremely practical question and upon which rests, in a large measure, the usefulness of the vaccine. If it is made necessary to dissolve this dried vaccine in water before it can be injected, the method will have defeated its own purpose. To round up several hundred head of cattle, many of them in a wild state, with the intention of injecting them with a liquid vaccine by means of a syringe and a frail needle, is a difficult task. This is recognized as a great disadvantage to Pasteur's vaccine and the spore vaccine in liquid form, which is being used at present. The spore vaccine should not only be desiccated, but it should be so prepared and standardized that it can be injected in the dry state in proper and safe proportions. This can readily be accomplished by moulding the dry spore vaccine into either the pellet or thread form, preferably the pellet, which insures the most convenient and the safest method for handling and injecting.

103805

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THE PREPARATION AND STANDARDIZATION OF OVARIAN AND PLACENTAL EXTRACTS.

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Anyone who has read the various articles and monographs that have appeared in the literature in the last few years on the internal secretion of the ovary or placenta has no doubt been impressed by the great disparity of results obtained with extracts of these organs. This also obtains for the other glands that possess an internal secretion with exception of the adrenals. One investigator will arrive at seemingly beautiful results with an extract of a certain endocrine gland, while another will obtain a result exactly opposite with an extract of the same gland. One investigator may isolate from the same gland two products, whose action is antagonistic. Another research worker will try to confirm the work of these others and obtain negative results, if indeed he does not get results different from those he is attempting to duplicate. The reason for this difference is that each one has made an extract by a different method and in a different way.

The need, therefore, for more uniform methods in the preparation of ovarian and placental extracts at once becomes apparent. For in no other way can the results of the different research workers and clinicians be compared. The tangible data, both in the laboratory and in the clinic, are at best small. At present results obtained from extracts of the entire ovary or from the yellow body alone, without note of whether aqueous, alcoholic, or ethereal extraction is employed, are reported. The results will of course vary and are not comparable. All these differences and annoyances will disappear as soon as the active principle of the ovary and placenta is isolated. But until then it would be much better if some uniform method for the preparation and the standardization of ovarian and placental extracts were formulated.

Perhaps a short review of some of the more important articles on the preparation of ovarian and placental extracts, and a description of the attempts made to isolate their active principle, may be of aid in showing the absolute necessity of a more uniform method of preparation. It is only within the last ten years that an attempt has been made to isolate the active principle of the ovary and placenta, especially the former. Before that and even up to the present time, the desiccated product of the ovary or corpus luteum has been given to allay symptoms referable to some disturbance of the internal secretory mechanism of the ovary. Among the first to attempt to make an extract that would contain the active principle of the ovary was Iscovesco. He investigated, as early as 1908, the action of the so-called "lipoids" obtained from the red blood corpuscles, the hypophysis, the kidney, the suprarenal capsules, the ovaries, the testicles, and the corpora lutea. He found that the "lipoids" obtained from these sources exerted a certain action on the female genitalia and discovered at the same time that there were two classes of these lipoids, classified according to their action. One, which he called "homo-stimulating," had an action on the same organ from which it was derived, and the other, called "hetero-stimulating," had an action on different organs. This division was purely arbitrary, as he discovered later.

Of special importance are the two "lipoids" which he characterizes as "II F. b." and "V. D. c.," both being obtained from the ovary; the latter he was able to isolate from the corpora lutea. His technique in preparation as given in his paper, which appeared in the Comptes Rendus de Soc. de Biol., 1912, lxxii, 858, is, briefly, as follows: The fresh organ is finely pulped and the pulp thrown into three times its volume of 95 per cent. alcohol, in which it is allowed to remain two hours. Then it is filtered, set aside to extract the lipoids and is designated as Fraction I. The residual pulp is desiccated at 40 degrees, pulverized and treated with different solvents. First, it is extracted with sulphuric ether and this is called Fraction II of the "lipoids." The residue therefrom is dried, powdered and treated with acetone in the same manner, which gives Fraction III. Further extraction with the resulting residue gives Fraction IV, and, lastly, with alcohol, Fraction V is obtained. All these fractions being prepared, their subdivision and purification follows. For example, with Fraction II the ether extract is dissolved in petroleum ether, giving an insoluble portion A, which is small in amount, and a soluble portion B, which constitutes the major part. This latter is desiccated and redissolved in ether. There remains then an insoluble part, which is removed by the centrifuge. The clear supernatant solution is then thrown into about ten times its volume of acetone, pure and free from any acid, and allowed to stand in the refrigerator 24 hours. At the end of this time an abundant precipitation will be found in the bottom of the receptacle, which, when filtered, constitutes portion C. The acetone solution is concentrated and put into the refrigerator, giving, after cooling, a yellowish white mass, which is portion D. The remainder of the acetone solution, which has passed through the filter, is then completely dried and the precipitate is dissolved in boiling alcohol. All should dissolve, after which it is cooled for 24 hours, giving portion E, and a dissolved portion F. Each of these fractions (A, B, C, D, E, and F), thus isolated, is far from being considered as chemically pure and each must be dissolved in petroleum ether, ordinary ether, cold alcohol, and finally in warm alcohol. There is obtained by this means the series a, b, c, d of the corresponding substances. The same procedure is gone through with Fractions III, IV and V. Each fraction was tested upon animals, and only those which produced a rapid growth of the uterus were considered "active."

Thus it will be seen from the careful and painstaking work of Iscovesco that when an extract of the ovary, corpus luteum or of the placenta is used in laboratory experiments or in the clinic, care must be exercised in obtaining the active fraction of the extract or the vital part of the organ may be left behind on the filter paper and thrown away. Concerning the physiological action of these fractional extracts of Iscovesco and the results obtained by him in the laboratory and in the clinic, no mention will be made. Those interested in this part of his work may consult his recent articles in literature.

According to his technique, outlined above, the "active substance," which he describes as "II F. b.," might briefly be considered as an ether extract of the desiccated organ (ovary), soluble in acetone (at ordinary temperature), having further been purified by boiling alcohol, concentration and extraction with petroleum ether. The other substance, V. D. c., appears to have been obtained from the residue remaining after treating first with alcohol, then with ether, then with acetone, followed by chloroform, and finally with alcohol again. This alcohol soluble substance is then presumably purified by solution in petroleum ether, resolution in ether and precipitation by acetone and in turn further purified by solution in petroleum ether.

ordinary ether and cold alcohol. The substance then is acetone insoluble, whereas his previous substance II F. b. is soluble in acetone.

Herrmann followed the plan of Iscovesco in the preparation of ovarian and placental extracts and published his article early in 1915. Herrmann believes that he has succeeded in separating the "active substance" of the corpus luteum and of the placenta as a specific chemical substance. He attempted to prove this clinically and on animals. Many colored figures are appended to his article and his results, if true, show wonderful changes from only a few doses of his so-called "active principle."

His method of extraction, while not identical with that of Iscovesco, given above, will not be described *in toto* at this time. He was able to obtain three fractions by his method, and these were further purified by distillation and tested. It was found that the so-called middle run produced a thickly viscous body, which represents, as he believes, the real active principle of the ovary. This active substance, as further described by Herrmann, is a yellowish glistening oil, which is solidified by cooling, but otherwise remains thickly viscous. It gives a very decided cholesterin reaction. It becomes brown when exposed to the air, apparently by taking up oxygen, and is composed of three elements, carbon, hydrogen, and oxygen. The body is a cholesterin derivative. It is soluble in alcohol, ether, petroleum, ether, acetone and benzol, but is insoluble in water.

Herrmann found the same active substance with identical physiological properties in the placenta, as well as in the corpus luteum. However, one placenta contains more of the active substance than one corpus luteum. The method of preparation is the same in both cases.

Seitz, Wintz and Fingerhut found that the corpus luteum contained two "bodies." One, which they designated as "luteo lipoid," has an inhibitory action on the blood and, injected subcutaneously, before and during the menses, lessens the flow and shortens it. The other body they call "lipamin," soluble in water, a luteo lipoid or lecithin-albumin, which causes, when injected into animals, an increased growth of the genitalia. Subcutaneously injected in women, it causes the appearance of the menses in cases of amenorrhea. "Luteo lipoid" and "lipamin" are antagonistic bodies and regulate the course of the menses. Their method of extraction is given in an indefinite sort of way. They obtain the various lipoid fractions by extraction with alcohol, chloroform, ether and acetone. By later evaporation, redissolving and extraction they obtained the two antagonistic bodies mentioned above.

A hasty review of the methods of these research workers (Iscovesco, Herrmann, Seitz *et al.*) shows that they all used practically the same method of extraction and, as far as can be gleaned from their clinical and experimental reports, they arrived at about the same results. However, the difference may be due to the fact that Herrmann obtained his so-called active substance in a purer state. These investigators at least used a certain definite method of extraction and their resulting products could be tested experimentally and clinically with a view of producing certain definite physiological and anatomical results.

Other workers, who have reported results obtained with ovarian or placental extracts, have been too prone to give the clinical results secured with, what they loosely call, an "extract." This may be an aqueous, alcoholic or ethereal extract or, as often happens, it is not an extract at all but simply the dried gland in the form of a desiccated powder. It should be clearly borne in mind that the kind of extract must be mentioned if the work is to prove of any value to others. A qualifying adjective such as "ethereal," "aqueous," if properly employed, may sufficiently designate the method of preparation. Not only is uniformity of preparation desirable, but some form of standardization of the resulting products is necessary to obtain extracts of the same strength and action. Many of the failures to produce the desired effect are due to a lack of proper standardization of an otherwise perfect product.

In order to perfect a proper preparation and later a suitable method of standardization, some research work was undertaken by the writer during the last two years. Up to the present time it can definitely be stated that no ideal method of preparation has been formulated and, until that is accomplished, the standardization of the product will not be attempted. Various forms of extracts of ovary, of corpus luteum, of the ovary minus the corpus luteum (ovarian residue) have been made by exact chemical methods and the resulting extract tried on animals. The results have all been negative. One extract, a powder soluble in water, has been obtained and clinically it has seemed to be active in some conditions, *i. e.,* disturbances of the artificial or the physiological menopause. But, in this connection, the results are too vague, ephemeral and transitory; hence conclusions drawn therefrom do not have much value.

For example, a few ampoules of the soluble extract are given or sent to Doctor A., with the request that he try its action upon the disturbances of the menopause when a suitable case presents itself. Later Doctor A. has a patient who "says" that she is suffering from "flashes of heat," "insomnia," etc. Doctor A. makes an examination and finds no pelvic pathology. But upon the statement of the patient he injects an ampoule of the soluble corpus luteum. In a day or two he repeats the injection. After a few injections Doctor A. "asks" the patient if she is feeling better, and the patient, not wishing to offend Doctor A., or thinking that she does feel better, answers in the affirmative. Doctor A. continues the treatment and later reports this case as one cured.

Results, based upon the statement of the patient, must be considered with extreme care. Yet that, practically, today is all the data upon which judgment can be passed as to whether a preparation or extract of the ovary has any therapeutic action. There are many factors in the preparation of an extract which may have an important bearing upon its therapeutic activity, such as the freshness of the material, the purity of the chemicals, proper temperature, avoidance of very high or very low degrees of heat or cold. Variations in all or any of these may tend to render the resulting extract inert or cause it to produce results that are due to faulty preparation.

In this connection mention will be made of Rosenheim, who reported his work on the pressor principles of placental extracts. Rosenheim was attempting to confirm the work of Dixon and Taylor, who claimed that alcoholic extracts of human placenta contained substances which, on intravenous injection, produced a rise of the blood-pressure and a contraction of the pregnant uterus.

Dixon and Taylor's method of preparing these extracts consisted in mincing fresh human placenta, covering it with absolute alcohol and filtering after some time. The filtrate was evaporated and again taken up with absolute alcohol. The residue of the last extract, dissolved in saline solution, was injected intravenously. Rosenheim thought that the effect of the injections seemed to suggest the presence of adrenalin or a similar substance. Trying to prove this, he found that the active principle was not identical with adrenalin. Rosenheim made alcoholic extracts in several ways, and found that the normal human placenta did not contain any pressor principle. Next he made three different extracts, as follows: In the first the fresh minced placenta was barely covered with alcohol to allow putrefaction to take place; in the second the fresh minced placenta was shaken up with one liter of saline solution saturated with chloroform and kept for four days at room temperature; and in the third, the fresh minced placenta was mixed with absolute alcohol (1000 c.c. of minced material to one liter of absolute alcohol). Rosenheim found that the first extract (above) contained a strong pressor principle, while the second and third did not, or, to quote his table:

	CONDITIONS OF EXPERIMENT.	RESULT.
A.	(No. 1 above) Extract after free putrefaction.	Strong pressor effect.
B.	(No 2 above) Extract after autolysis alone.	Total absence of pressor effect.
C.	(No. 3 above) Extract of fresh organ.	Total absence of pressor effect.

Rosenheim was later able to identify this pressor principle of Dixon and Taylor as belonging to the amines and it probably is derived from the amino acids (leucine and tyrosine), the cleavage products of proteins.

Barger and Walpole came to practically the same conclusions in their work upon putrid meat, *i. e.*, that it contains a pressor principle.

Five extracts of the placenta were prepared by the writer and their action tested on the blood-pressure and upon the isolated uterus, as well as by injection into animals (rabbits). These extracts were prepared as follows:

Number one was an aqueous extract obtained by exposing the minced placenta to distilled water, slightly acidulated. The resulting fluid was boiled with acetic acid to precipitate the coagulated proteins, filtered, evaporated and distilled water added to bring it up to its original volume. This solution was passed through a Berkfeldt filter and filled into 1 c.c. sterile ampules.

Number two was obtained by placing in a wide-mouthed bottles 725 gm. of minced placenta pulp. This mixture was allowed to stand at room temperature for four days, loosely corked. Then 725 c.c. sterile distilled water added, mixed and filtered and the filtrate boiled with acetic acid. This was filtered while hot, and the filtrate cooled and evaporated to more than one-half its volume. Sterile distilled water was added to bring the amount up to 725 c.c., the original volume. This latter was next passed through a Berkfeldt filter and filled into 1 c.c. sterile ampules.

Number three was prepared by taking 400 gm. of minced placental pulp and adding to it 400 c.c. of normal salt solution, saturated with chloroform. This was loosely corked and allowed to stand at room temperature for four days. It was then filtered and the filtrate boiled with acetic acid to precipitate the coagulated proteins. This was again filtered while hot, cooled and evaporated to less than one-half its volume. Sterile distilled water was added to bring the amount up to 400 c.c., the original volume. It was then passed through a Berkfeldt filter and filled into 1 c.c. sterile ampules.

Number four was prepared by adding 400 c.c. of absolute alcohol to 400 gm. of minced placental pulp. This was allowed to stand for some time at room temperature in bottle loosely corked. Then the whole was filtered, the filtrate boiled with acetic acid, filtered through paper and evaporated to less than half of its volume. Sterile distilled water was added to make 400 c.c., the original amount. This was passed through a Berkfeldt filter and filled under sterile conditions into 1 c.c. sterile ampules.

Extract number five was made by taking about 500 gm. of minced placental pulp, which was placed in a large-mouthed bottle. This was allowed to stand in the incubator, loosely corked, for several days. The mixture was shaken every two or three days. Then it was filtered and treated as the others (Nos. 1-4) and filled into sterile 1 c.c. ampules.

These five extracts were submitted to Mr. L. W. Rowe to be tested on the isolated uterus of the guinea-pig and upon the blood-pressure of a chloretonized dog. Mr. Rowe reported as follows: All five extracts contracted the isolated uterus of the virgin guinea-pig, when administered in sufficient quantity. About 0.25 c.c. of the undiluted sample was necessary in each case to produce an appreciable contraction. The action of number five (5) and number two (2) seemed a little stronger than that of the others, and this was due to the fact that these extracts were partly decomposed. All meat extracts will contract the isolated uterus if they are sufficiently concentrated, and this action is more marked if decomposition or partial decomposition has taken place.

The effect of these extracts upon the blood-pressure was not very marked nor characteristic. In most instances the blood-pressure fell slightly, but this was more marked with number five (5), which action was no doubt due to the very evident decomposition of this sample. The results of these tests indicate that placental extracts do not contain a pressor or marked oxytocic principle.

The action of these extracts (Nos. 1-5) upon animals, when injected intraperitoneally, was negative. A large number of injections produced no macroscopic effect upon the internal or external genitalia of the animals injected. There was no anatomical difference between them and the control animals.

From a review of the more recent literature, from the animal experiments thus far undertaken with extracts of the corpus luteum, ovary and placenta, and from the different methods employed in making these extracts, the following conclusions seem justifiable.

1. A more uniform method of preparing extracts must be instituted.

2. Some method for the standardization of these extracts must be discovered, in order to facilitate the comparison of the results of the different laboratory workers and clinicians.

3. Many results obtained in the laboratory or in the clinic are due to the faulty preparation of extracts.

4. The isolation of the active principle of the ovary and the placenta will clear up many, if not all, of these mooted points.

BIBLIOGRAPHY.

Barger, G., and Walpole, G. S. Isolation of the pressor principles of putrid meat, Jour. of Phy., London, 1909, xxxviii, 343.

Dixon, W. E., and Taylor, F. E. Physiological action of the placenta, Brit. Med. Jour., 1907, ii, 1156.

Herrmann. Ueber eine wirksame Substanz in Eierstocke und in der Placenta, Monatsch. für Geburtsh. und Gyn., 1915, xli, 1.

Iscovesco, H. Etudes sur les lipoides de l'organisme, etc., Compt. rend. Soc. de biol., Par., 1907, lxiii, 744.

Iscovesco, H. Les lipoides des globules rouges du sang. Preparation, etc., Compt. rend. Soc. de biol., Par., 1908, lxiv, 269, 324, 404, 548, 675, 677.

Iscovesco, H. Les lipoides, Presse Med., Par., 1908, xvi, 457, 529 and 553.

Iscovesco, H., *et al.* Sur les lipoides solubles dans l'ether et insolubles dans l'acetone de quelques orgnsa, etc., Compt. rend. Soc. de biol., Par., 1908, lxv, 217. 218.

Iscovesco, H. Les homostimulines: étude experimentale et clinique du lipoide utero-stimulant du rein et du lipoide du corpe junea, Bull. et mem. Soc. Med. d. hôp de Paris, 1912, 3. s. xxxiv, 106, 758.

Iscovesco, H. Le lipoide utero-stimulant de l'ovaire; proprietes physiologiques., Compt. rend. Soc. de biol., Par., 1912, lxxiii, 16, 104 and 189.

Iscovesco, H. Les lipoides de l'ovaire, du corp jaune et du testicule; proprietes homo-stimulates, physiologiques et therapeutiques, Presse Med., Par., 1912, xx, 845.

Iscovesco, H. The lipoids of the ovary; corpus luteum and testicle, Univ. M. Rec., Lond., 1912, ii, 395.

Iscovesco, H. Lipoides homo-stimulants de l'ovaire et du corps jaune; étude physiologique et therapeutique, Rev. de gynec. et de chir. abd., Par., 1914, xxii, 161.

Rosenheim, O. The pressor principles of placental extracts, Jour. of Phy., London, 1909, xxxviii, 337.

Seitz, L., *et al.* Ueber die biologische Funktion des corpus luteum usw., Münch. Med. Wochensch., 1914, lxi, 1657 and 1734.

103813

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PARASITES OF THE DOG IN MICHIGAN.*

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While the parasites of the dog are of general interest, they are of particular interest to the veterinarian. The dog is one of the animals which come to our attention as patients, and parasitic infestations constitute a number of the complaints we are called on to treat. In our scientific work they serve as admirable experiment animals, and their response to treatment is so similar to the human response that our findings in regard to dogs are very readily applied to similar conditions in man; the dog has a digestive tract, food habits, responses to drugs and emotional responses very similar in most respects to those in man. Finally, dogs have certain good and certain bad qualities which concern us as individuals. Their good qualities make them valuable as companions, hunters, sheep dogs, watch dogs, etc. Their bad qualities make them objects of suspicion or condemnation as sheep killers, as destroyers of flower beds, as defilers of streets, sidewalks, and even foodstuffs, and as carriers of parasites and disease.

That the dog is of real importance as a carrier of parasites and disease is something that is only beginning to be realized. Of late we have been centering our attention on the louse, the fly and the mosquito as carriers of disease, but we must realize, and doubtless will in time, that the fly's capacity for carrying trouble into the homes of careful and intelligent people is probably less than the capacity of the dog who leaves that home by day or night to wander in Heaven knows what filthy and germ-laden surroundings, to consort with other dogs, themselves diseased, and from homes where there are diseased persons and diseased animals, and finally to come home to cuddle up on the foot of the children's bed, to take a nap on the couch pillows, where presently the head of the dog's master or mistress or child playmate will rest, or to thrust his nose into a welcoming hand or a friendly face to receive or bestow caresses. We have learned to welcome the fly with a swatter; perhaps we will learn to welcome our pet dog, after he has been out for a day's hike or a night's debauch, by prompt immersion in a bath of adequate germicidal and insecticidal character. The rat and the rat-flea, the combination that keeps the human race in danger and dread of bubonic plague, are being met with rat-proof buildings, rat-killing campaigns, and rat guards and fumigation for ships, and legislators are making liberal appropriations to carry out these measures. Fortunately, this campaign meets with no opposition from any body of persons claiming to be lovers of rats. Unfortunately, the campaign to put the vagrant, worthless cur and the carrier of rabies in the same category as the typhoid fly and the plague-bearing rats meets with a considerable opposition from persons who love good dogs, poets who write verses to the constancy of the yellow cur, and zoophiles who would sacrifice the welfare of the human race to conserve the supposed welfare of the lower animals.

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How general is the growing interest in the dog as a carrier of disease may be judged from a few facts:

The British Medical Journal of July 15, 1916, had an editorial entitled "In Dirty Dogdom," which says in part: "The French Council of Hygiene devoted the greater part of its last session to the consideration of the public dangers associated with the inordinate keeping of dogs and the insufficient control exercised over them. In the first place, the Council, having discussed at length the * * * recrudescence of rabies * * * determined to require that the measures for holding the disease in check should be most strictly applied. Next, Professor Raphael Blanchard undertook to prepare a report on the dangers of dissemination of hydatid disease by dogs and the preventive means which should be taken. Finally, the Council adopted a resolution urging that the regulations with regard to the municipal taxes on dogs should be vigorously applied.

At about the same time, the Medical Council of the British Science Guild was adopting a resolution condemning the pollution of the streets of London and of most cities and big towns by dogs, and suggesting to the government and municipalities that the evil might be reduced by increasing the tax on dogs and enforcing by-laws * * * In towns the tax on dogs should be doubled, and a large progressive increase imposed on each additional dog. The proposal will probably have the cordial support of the majority of retail traders, who daily suffer loss owing to the primitive habits of the little darlings that the women drag about with them."

In this country the U. S. Department of Agriculture has issued a bulletin (Hall, 1915) dealing with the dog as a carrier of parasites and disease, and the sentiment of that bulletin, to the effect that dogs should be given reasonable care and supervision by their owners, or destroyed if they have no owners, has been editorially approved by the Journal of the American Medical Association and a number of other medical and non-medical publications. Similar papers, asking for a reasonable control of dogs, have appeared in various places, and among the latest papers of the sort is one in Public Health, published by the Michigan State Board of Health, for January, 1917, under the heading "When the dog becomes a nuisance." In the paper just mentioned, the author (Bush, 1917) says "Practically every dog is loaded with intestinal worms." Which brings us, finally, to our immediate topic, the parasites of the dog in Michigan.

What parasites of the dog are found in Michigan and to what extent are they present? These questions I expect to answer, so far as possible, principally from post-mortem examinations of 74 dogs from the Detroit city pound, covering the period from October 1, 1916, to February 3, 1917. I have made only a hasty examination of the literature along this line and find very little published covering the subject for the State of Michigan. While the parasites of dogs in Detroit for the season mentioned will give a fair idea of conditions in general, it must be borne in mind that there will be certain differences between city dogs and country dogs, and differences may be expected in degree or kind of infestations for summer and winter, north and south Michigan, and nearness to or remoteness from bodies of water.

Before taking up a consideration of the internal parasites of the dog, just a word in regard to the external parasites. There has been very little time for investigation of this sort, but, on casual observations of a few dogs, we have found one heavily infested with lice, both the biting louse, *Trichodectes latus,* and the sucking louse, *Linognathus piliferus* (*Haematopinus piliferus*), being present, the biting lice being much the more numerous. Osborn (1896) says of the sucking louse: "We have examined many dogs in quest of it, but only a single specimen so far has been our reward." Herms (1915), on the other hand, says: "Although other observers have found the sucking dog louse, *H. piliferus* Burm., less common than the biting dog louse, the writer has found this species quite as common in California, if not relatively more abundant." Prof. Pettit, Entomologist at the Michigan Agricultural College, tells me that he has not found lice at all common on dogs in Michigan, the biting louse being seen more often than the sucking louse. We found one dog plentifully infested with the common dog flea, *Ctenocephalus canis,* and doubtless this flea is plentiful on dogs in Michigan as it is in most places. We did not find any ticks on dogs, nor any records of the sort, though Hooker, Bishopp, and Wood (1912) published a map of the United States on which Michigan is shown as within probable range of the common American dog tick, *Dermacentor variabilis.* Prof. Pettit states that hunters in Michigan have told him that they had found ticks on their dogs in this state, but no specimens have come in and the species is, therefore, uncertain. The demodectic mange mite, *Demodex folliculorum* var. *canis,* occurs in Michigan, as numerous cases of demodectic mange, reported to me verbally by Dr. Ferry and Mr. Rowe at Detroit, and by Dr. Ward Giltner and Prof. Pettit at Lansing, testify. Sarcoptic mange, due to *Sarcoptes scabiei* var. *canis,* probably occurs in Michigan as elsewhere, but I have not yet seen any cases or found any reports of this.

Regarding the internal parasites of the dogs examined, a few figures may be given. Of 102 dogs that came into our hands, 28 were rejected for experiment purposes on the strength of a negative fecal examination indicating that these dogs were not infested. This does not necessarily prove that these dogs had no intestinal parasites. Worm eggs are not always present in the feces even when worms are present in the intestines, for the worms may be too immature to pass eggs, or they may be all males (a condition I have seen a number of times), or the females may be so few (and we have several times seen a single female of a given species present) that the eggs are very scarce and are not found, or egg production may be inhibited by a number of possible conditions. Dogs with negative fecal examinations were occasionally accepted for experiments where infestation was unnecessary, and were later found to have light infestation. Of the 74 dogs examined post-mortem, 67, or 91 per cent, were found to have internal parasites. In other words, 67 dogs out of 102, or about 66 per cent, were certainly infected, and since some of the rejected 28 dogs would be found to have some internal parasites if examined postmortem, we can feel sure that, on an average, more than two out of three Michigan dogs are infested with internal parasites, so far as dogs from the Detroit city pound are indicative of conditions. On this point it might be said that the pound dogs are fairly representative. These dogs represent almost all breeds except the toy varieties, though of course nearly all of them are mongrel or at least not pure bred. Our records show the following: Mongrels, terriers, rat-terriers, collies, English bulldogs, hounds, poodles, bull-terriers, mastiffs, spaniels, cocker spaniels, dachshunds and mongrels of all these breeds.

Of the 67 infested dogs, 2 dogs, or 3 per cent, were infested with coccidia. So far, the forms I have examined, apparently the oocysts, have not been sufficiently developed to warrant even a tentative determination, but the large size, 36 to 40 μ long by 28 to 32 μ wide, precludes the idea that this is the form described from the dog in Europe by Stiles under the name of *Coccidium bigeminum (Diplospora bigeminum),* since the latter species and its varieties seem to attain a size less than half of this, so far as literature is available to me. Regarding the pathogenicity of these coccidia I can say little. One dog died 5 days after coming into our possession. This dog's liver was light colored, apparently due to some degeneration, but showed nothing suggestive of the condition present in hepatic coccidiosis of rabbits. The immediate cause of death was an intussusception of the ileum into the colon by way of the ileocecal valve, with a resultant hemorrhage that filled the cecum and colon with blood. Various coccidial stages were found in scrapings of the mucosa of the small intestine. The other dog showed a temperature of 101° to 101.5° and was bright and active for six days; the next day the temperature was 102.5°, the next, 98.8°, the next, 95°, falling to 94.5°; the next day the dog was found dead. The liver showed no evidence of coccidiosis, and the coccidian is evidently an intestinal form, so far as our evidence goes. This dog had been under experimental treatment to test the efficacy of treatments designed to kill the coccidia. The number of coccidia present in the feces did not appreciably diminish during the short period of treatment, but it appears from the gastro-enteritis found on postmortem that the treatment or the combined effect of the treatment and the coccidiosis killed the dog.

Of the tapeworms found in the dog at Detroit, the most common are species of *Dipylidium*. Of the 67 infested dogs, 31, or 46 per cent, were infested with *Dipylidium,* the worms being present in numbers from 1 to 205. One of these species is the form commonly reported, *Dipylidium caninum*. The other is *Dipylidium sexcoronatum*. This latter species has never been reported from the United States before. In Detroit, it seems to be more common than *D. caninum*. I have also found it in the dog at Bethesda, Md., near Washington, D. C. I venture to surmise that a careful examination of specimens of the double-pored tapeworm from dogs, commonly reported as *D. caninum,* will show a large proportion to be *D. sexcoronatum*.

It might be noted that *Dipylidium caninum* has been reported as a parasite of man about 80 times, 3 of the records being American. In one of these cases, reported by Stiles (1903), the worm was passed by a 16 months old child in Detroit.

Joyeux (1916) has recently demonstrated that fleas, *Ctenocephalus canis* and *Pulex irritans*, become infested with the intermediate stage of *Dipylidium* while in the larval stage, the adult flea being unable to ingest the egg of *Dipylidium*.

Of the 67 infested dogs, 4 dogs, or 6 per cent, were infested with tapeworms of the genus *Tænia*. In two cases there was 1 specimen of *Tænia hydatigena (T. marginata)* present, indicating that the dogs had fed on offal of sheep, cattle or swine infested with *Cysticercus tenuicollis,* the thin-necked bladder-worm commonly found in the omentum and mesenteries of these animals. In the other two cases there were, respectively, 1 specimen and 3 specimens of *Tænia pisiformis* (*T. serrata*), indicating that the dogs had fed on offal of rabbits infested with *Cysticercus pisiformis,* the bladder-worm commonly found in the body cavity of rabbits. Prof. Pettit has specimens of this parasite collected from the cottontail rabbit, probably *Sylvilagus floridanus mearnsii,* around Lansing, and I have seen the same parasite in the cottontail, probably the same species, purchased in the market at Windsor, Canada, opposite Detroit. This bladder-worm and its tapeworm are probably common in Michigan as they are in most places. I noted (Hall, 1910) in a paper on the genus *Multiceps* (the tapeworms having a cœnurus, a thin walled bladder-worm with numerous heads instead of one, for an intermediate stage) that there was in the collection of the Bureau of Animal Industry, at Washington, D. C., a specimen of *Multiceps serialis* collected from the rabbit in Michigan in 1904. This is the species which occurs as a cœnurus in the connective tissue of the rabbit and other rodents, sometimes forming large swellings under the skin. The corresponding tapeworm occurs in the dog, and it is probable that this species occurs in the dog in Michigan.

Regarding the presence of other dog tapeworms in the State of Michigan, I have no positive information, but there are some surmises that may be made.

The gid tapeworm, *Multiceps multiceps (Tænia cœnurus),* has never been recorded from Michigan. The larval stage, or cœnurus, of this worm was reported from Michigan in the American Shepherd's Bulletin in 1903, the veterinary editor diagnosing a case in two imported rams on the very characteristic symptoms given by a correspondent. Everything indicates that the disease was imported and that Michigan has been and is free from gid.

Dr. Shafter of the U. S. Bureau of Animal Industry at Detroit tells me that hydatids, the thick-walled echinococcus cysts, are occasionally found in meat inspection of swine which may be of Michigan origin. Should these hydatids be found to occur in Michigan hogs, it would indicate that the corresponding tapeworm giving rise to this very important larval tapeworm is present in Michigan.

The broad tapeworm, *Diphyllobothrium latum* (*Dibothriocephalus latus*), which is a parasite of dogs as well as of man, has been reported from man in Michigan by Hagelstam (1896).

His case occurred in a native of Finland and was probably imported. However, this parasite has been found in persons who have never been outside of the United States, and Nickerson has found the larvæ of this worm in fish caught in the Great Lakes. It is, therefore, quite possible that this tapeworm may be found, sooner or later, in Michigan dogs.

As regards the flukes in Michigan dogs, I do not find any records. However, the lung fluke, *Paragonimus kellicotti,* which occurs in dogs, cats and hogs in the United States, has been reported, under the name of *Distoma westermanni* (*Paragonimus westermanni*), from the cat in Michigan by Ward (1894). It is quite likely that this fluke occurs at times in Michigan dogs.

Among the nematode parasites of the dog in Michigan, the commonest is the ascarid. Of our 67 infested dogs, 47, or 70 per cent, were infested with from 1 to 100 ascarids. In other words, of 102 dogs, 47, or 46 per cent, were infested with ascarids on the basis of post-mortem examination or fecal examination. Allowing for the limitations of fecal examination, it may be confidently asserted that half of the dogs in Michigan are infested with ascarids, if the Detroit figures are indicative of conditions, as they probably are. This is a higher figure than I find reported for Denmark, Iceland, Australia, Germany, or elsewhere in this country.

All of the worms that I have examined have been *Belascaris marginata,* which is also the common form at Washington, D. C. The other species, *Toxascaris limbata,* is apparently the less common form in this country. These worms are normally parasitic in the small intestines, but are occasionally found in the stomach, a condition which commonly leads to their being vomited. They are an important pest of dogs, and are often held responsible for fits in pups. It is quite likely that they are often responsible, as these large active worms in the intestines give rise to considerable irritation and at times quite surprising reflexes. Ascarid worms are notorious for their wandering habits, often entering the bile duct, pancreatic duct, cecum, and stomach, and occasionally coming up the esophagus, and down the trachea, or into the eustachian tube. These conditions, commonly reported for man and for hogs, are apparently rare in dogs, and I have never seen the ascarid outside of the stomach or small intestines, except when passing out via the large intestine. One of these ascarids, *Toxascaris limbata,* has been reported a number of times from man, due to too friendly intimacy between man and dog and disregard of hygienic precautions.

Galli-Valerio (1915) says that he has confirmed the work of Dorbernecker in finding evidence of blood in ascarids, the blood being demonstrated by spectroscopic methods. He regards this as evidence that ascarids are blood suckers. This is not wholly conclusive. According to Garin, ascarids feed on epithelial cells, and the evidence of common observation and of the morphology of the ascarid's mouth should absolve it from the claim that it is a blood sucker in the ordinary sense of the word. Recently an ascarid which I collected from the feces of a dog showed a pronounced red color in the intestine, evidently due to blood. But a postmortem examination of the dog the same day showed the presence of a severe hemorrhagic enteritis. This was evidently the explanation for the blood in the ascarid in this case. Something of the same sort may have been the explanation in the cases of Dorbernecker and Galli-Valerio. In other words, ascarids will doubtless ingest blood as well as epithelial cells or other things, without being true blood suckers. The ascarids may occasionally lacerate the intestinal mucosa, and we know that arcarids occasionally perforate the intestinal wall, and in so doing it is likely that they ingest some blood. But this must be regarded as exceptional; and before regarding the ascarid as a blood sucker, it should be demonstrated that ascarids in general show the presence of host blood.

In another paper (Hall, 1917) I have recorded some experiments on the longevity of ascarids outside of the body of the host. Wharton (1915) kept the ascarids of man, *Ascaris lumbricoides,* alive in Kronecker's solution (normal salt solution to which 0.06 gram of sodium hydroxide per liter is added) for 6 to 12 days. At Detroit we have kept dog ascarids, *Belascaris marginata,* alive in this solution for 14 days. The ascarid of the pig, *Ascaris suum,* I have kept alive in Kronecker's solution for 26 days, and in normal salt solution for 15 days. In the paper mentioned, I have pointed out the bearing of these facts on the action of anthelmintics. It is believed by many veterinarians, physicians and laymen that when a worm-infested patient is fasted for 12 to 24 hours, the worms become hungry and will eagerly ingest an anthelmintic, especially if administered in what is regarded as a palatable vehicle. It will be evident that a worm that can live 2 or 3 weeks or longer on such an innutritious diet as Kronecker's solution, will suffer little for 12 hours spent in feeding on its customary diet, the epithelial cells, in the warm intestinal tract of its host. Of course, the fasting preliminary to anthelmintic treatment is valuable since it removes much of the bulky food that might protect the worm.

Next in number of infestations to the ascarids are the whipworms, in numbers from 1 to 33. They are the whiplike worms which occur in the cecum usually, with the slender anterior end sewed into the mucosa. It has been supposed that the penetration of this unarmed head into the mucosa is effected by the solvent action of some secretion. Its pathological importance in dogs does not appear to be very great as a rule. The whipworm, *Trichuris depressiuscula,* was present in 28, or 42 per cent, of our 67 infested dogs.

The next most common parasite in our series of dogs was the hook-worm, *Ancylostoma caninum.* This was present in 23 of our 67 infested dogs, or in 34 per cent. There were from 1 to 70 present. I have never yet seen a specimen of the other dog hook-worm, *Uncinaria stenocephala,* nor were there any specimens in the extensive collection of the U. S. Bureau of Animal Industry at Washington, but Muldoon (1916) reports both hookworms as present at the clinic of the College of Veterinary Medicine at Ithaca, N. Y.

So far I have seen no hookworm infestations in Michigan dogs which were as heavy as those in Washington, D. C. The heaviest infestation seen in Detroit totaled 70 hookworms, and this infestation in a puppy had given rise to a clinical case of hookworm disease. In Washington, Mr. W. D. Foster and I found infestations with 104, 233, 242, and 812 hookworms. This is what might be expected. The development of hookworm eggs outside of the host animal to the infective stage depends largely on the factors of warmth and moisture. There is an abundance of moisture in Michigan, as there is in the District of Columbia, but the lower temperatures in Michigan are less favorable to hookworm development than are the higher temperatures of the

District. In the southern United States conditions are even more favorable to hookworm infestation, and in some localities breeders find it difficult to raise pups on account of the mortality from this disease.

Of the nematode parasites of the dog, one which does not occur in the digestive tract has lately received some little attention in print. This is *Dioctophyme renale* (*Eustrongylus gigas*), the giant kidney worm of the dog. This worm is the largest of all the true nematodes, the female attaining a length of about one meter and a thickness of over a centimeter. The worm is blood red and very striking in appearance. It is usually reported from the kidney or from the body cavity, rarely from other locations. Riley (1916) recently summarized the cases from the United States and Canada, a total of 27 cases, the worms being in the body cavity in 12 cases or 44 per cent. From Riley's paper it appears that cases have been reported from Charleston, S. C., Georgetown, S. C., Washington, D. C., Baltimore, Md., Pittsburgh, Pa., Philadelphia (?) Pa., Ithaca, N. Y., Albany, N. Y., New York City, N. Y., Kingston, Ont., Toronto, Ont., Columbus, O., and Winnipeg, Manitoba. A case which Leidy reports, on the authority of Mr. Joseph Jones, of Georgia, of the occurrence of the worm in the heart of a dog, has been generally discredited by parasitologists, though Riley is inclined to accept it. Personally, I feel that there was more likelihood of mistaking a blood clot for this worm, and a mistake of this sort is known to have been made, than that such an unusual thing should be true.

As I noted that Riley's records did not include two published cases of which I had records, I published a note (Hall, 1916) reporting these cases and adding a new case. One of the published cases was from Baltimore, Md., and the other, as a later note by Kaupp (1916) makes clear, from Chicago, Ill. The new case was from Ann Arbor, Mich., and is the first record of this parasite from Michigan. To that record I wish to add at this time. Two of our 67 infested dogs from the Detroit pound, or 3 per cent, were infested with *Dioctophyme renale*. In one case the worm was a female, 36⅜ inches (91.7 cm.) long by 5/16 inch (8 mm.) thick. The head was near the gall bladder, the worm extending forward between the lobes of the liver on the right side, then forming a large coil between the liver and diaphragm to the left side and along the body wall, then in to the intestines, the tail lying under the omentum. In the other case there were two male worms present in the abdominal cavity, one lying between the body wall and the liver and intestines on the right side, the other involved in the gastro-hepatic omentum. In both dogs the great omentum was inflamed. This is a common lesion in infestation with this worm, and presumably results from the protective activity of the omentum in guarding the host animal from the excretions and secretions of the parasite. Dr. Brenton tells me that he has seen about a half-dozen cases of the occurrence of this worm in dogs in Detroit, the worm being in the body cavity in all cases. My own records added to Riley's show a total of 32 cases from the United States and Canada, 3 of these cases being from Michigan. Of the 32 cases the worm was found in the abdominal cavity in at least 16 cases, or 50 per cent. With Dr. Brenton's cases we have about 38 cases from the United States and Canada, with over half of the records reporting this giant kidney worm from the abdominal cavity.

(Since the above was written, a later paper by Riley (1917) has come to hand. In this paper Riley records one additional case from Chicago, and notes an indefinite number of cases at the same place. It appears, therefore, that the worm has been found at least 40 or 50 times in the United States. Riley discusses the theory, first suggested by Stratton in 1843, that infestation of the peritoneal cavity might occur by way of the Fallopian tubes in bitches. In the American records, 7 of 8 peritoneal infestations, where the sex is given, were in females. In both of my cases the animals were females, making a total of 9 cases out of 10, or 90 per cent of cases in females for American cases where the sex is known.)

It is rather surprising to note that in our series of necropsies cases of infestation with *D. renale* were as numerous as cases of infestation with coccidia or even with the very common *Tænia hydatigena* or *T. pisiformis*. It is surmised that *D. renale* has an intermediate stage in fish, since it occurs in the seal, otter, mink, etc. If this is true, it may account in some degree for the surprising frequence of these worms in Detroit.

A comparison of the figures for the 67 infested dogs examined by Mr. Drake and myself in Detroit with those for 76 infested dogs examined by Mr. Foster and myself at Washington, indicates that in general worm infestations are slightly more numerous and heavier in Washington. Thus 51 per cent of Washington dogs have *Dipylidium* in number from 1 to 1500; 8 per cent have *Tænia* in number from 1 to 2; 57 per cent have whipworms in number from 1 to 331; 71 per cent have hookworms in number from 1 to 812. On the other hand, 70 per cent of Detroit dogs have ascarids, while a slightly smaller proportion, 67 per cent, of Washington dogs are so infested, and 3 per cent of our series at Detroit had *Dioctophyme,* while we found it in none of the larger series at Washington. At an earlier period in Washington, Sommer (1896) found *Tænia* in 14 per cent; *Dipylidium* in 44 per cent; ascarids in 28 per cent; hookworms in 56 per cent; whipworms in 70 per cent; and *Dioctophyme* in 2 per cent. It would appear from this that Washington dogs have more hookworms and whipworms, while Michigan dogs have more ascarids. The above figures cannot be compared exactly for the reason that we had rejected some dogs on negative fecal examinations.

Dogs in Lincoln, Nebraska, according to Ward (1897), have few hookworms and ascarids, and many tapeworms. In Colorado Springs, Colo., I found hookworm infestations quite uncommon, doubtless due to the combination of bright sunshine and dry air and the low temperature at night.

BIBLIOGRAPHY.

Bush, A. H. 1917. When the dog becomes a nuisance. Public Health, Lansing, Mich., N. S., v. 5 (1), Jan., pp. 20-22.

Galli-Valerio, Bruno, 1915. (Blood in ascarids.) Centralb. f. Bakteriol., Parasitenk. (etc.), Jena, v. 76 (7), Aug. 25. (Not available; review seen.)

Haglestam, Jari. 1896. Anemia caused by tapeworm (Bothriocephalus latus) observed in the United States. N. Y. M. J., v. 64, Aug. 29, pp. 289-291.

Hall, Maurice C. 1910. The gid parasite and allied species of the cestode genus Multiceps. I. Historical review. Bu. Anim. Indust. Bull. 125, pt. 1, 68 pp., 1 fig.

1915. The dog as a carrier of parasites and diseases. U. S. Dept. Agric. Bull. 260, 27 pp., 14 figs.

1916. American records of Dioctophyme renale. J. Amer. Vet. M. Ass., n. s., v. 3 (3), pp. 370-371.

1917. The longevity of ascarids outside of the body of the host and its bearing on the action of anthelmintics. J. Amer. M. Ass., v. 68 (10), Mar. 10, pp. 772-773.

Herms, William B. 1915. Medical and Veterinary Entomology. N. Y., 393 pp., 228 figs.

Hooker, W. A., Bishop, F. C. and Wood, H. P. 1912. The life history and bionomics of some North American ticks. U. S. Bu. Entom., Bull. 106, 239 pp., 17 figs., 15 pls.

Joyeaux, Ch. 1916. Sur le cycle evolutif de quelques cestodes. Note preliminaire. Bull. Soc. Path. Exot., v. 9 (8), Oct. 11, pp. 578-583.

Kaupp, B. F. 1916. Dr. Kaupp identifies a rare parasite found in the peritoneal cavity of a dog. Alpha Psi Quarterly, v. 2 (3), Sept., pp. 6-8.

Muldoon, W. E. 1916. Uncinariasis in dogs. Rept. N. Y. St. Vet. Coll. for 1914-'15, pp. 136-141.

Osborn, Herbert. 1896. Insects affecting domestic animals: an account of the species of importance in North America, with mention of related forms occurring on other animals. U. S. Div. Entom. Bull. 5, n. s., 302 pp., 170 figs., 5 pls.

Riley, William A. 1916. The occurrence of the giant nematode, Dioctophyme renale (Eustrongylus), in the United States and Canada. J. Amer. Vet. M. Ass., v. 49 (6), Sept., pp. 801-809.

1917. Another case of the occurrence of the giant nematode, Dioctophyme renale, in the abdominal cavity, and data bearing upon the theory of entry via the genito-urinary tract. Cornell Vet., v. 7 (1), Jan., pp. 43-45.

Riley, William A., and Chandler, W. L. 1916. The occurrence of the giant nematode on the liver of a dog. Cornell Vet., v. 6 (4), Oct., pp. 209-212, 2 pls.

Sommer, H. O. 1896. Results of an examination of fifty dogs, at Washington, D. C., for animal parasites. Vet. Mag., Phila., v. 3 (8), Aug., pp. 483-487.

Stiles, Ch. Wardell. 1891. Note preliminaire sur quelques parasites. Bull. Soc. Zool. de France, v. 16 (6), June, pp. 163-165.

1903. A case of infection with double-pored dog tapeworm (Dipylidium caninum) in an American child. Amer. Med., v. 5 (2), Jan. 10, pp. 65-66, 4 figs.

Ward, Henry B. 1894. On the presence of Distoma westermanni in the United States. Vet. Mag., Phila., v. 1 (5), May, pp. 355-357.

1897. Report of the Zoölogist. Animal parasites of Nebraska. Ann. Repr. Nebr. Bd. Agric. (1896), pp. 173-189, figs. 1-12.

Wharton, Lawrence D. 1915. The development of the eggs of Ascaris lumbricoides. Philippine J. Sci., Sec. B., Trop. Med., v. 10 (1), Jan., pp. 19-23.

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PREPARATION OF PROTEIN EXTRACTS FOR DIAGNOSTIC CUTANEOUS TESTS.

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So much work is being carried on at the present time from a clinical standpoint, relative to anaphylactic skin reactions with various protein extracts, including the food, plant, pollen and microörganismal, that it has become an important and, in some respects, a serious question as to the most practical form in which to present them. This includes the best method of extracting the crude material, so that all of the available proteins are, according to Goodale[1] "in the form in which they exist when exciting symptoms," the most stable form in which to preserve them, and the most suitable and appropriate state in which to exhibit them for administration.

If the cutaneous method of diagnosticating the sensitizations to various proteins is to become of general clinical use, taking its place by the side of the tuberculin, the luetin and other tests, it is evident that the present forms in which the proteins are found should be modified.

In preparing protein extracts for use other than experimental, several things must be taken into consideration. The material, as has previously been mentioned, must be extracted in such a manner as to retain all of the proteins of the foods, plants, and pollen in an available concentrated state; the extract must be preserved from deterioration due to heat, chemical action, and bacterial contamination, and should be so mixed with a non-irritating soluble diluent that it can be used directly by the diagnostician.

There seems to be no dissenting opinion as to the value of water as an extractive, all authorities agreeing that the large majority of proteins are perfectly soluble in water, although it is recognized that such extracts are extremely liable to deterioration. To insure stability of the aqueous extracts, especially toward microörganismal contamination, which appears to be the most destructive influence, various preservatives are used and good results have been reported from all of them. Those most frequently employed are alcohol, glycerin and the phenols.

Goodale advises the use of alcohol in from 12 per cent to 15 per cent dilution, as he believes it best preserves the anaphylactogenic properties of pollen and other protein extracts. He found, however, that the pollen solutions contained one unstable albumen and a relatively stable proteose, the former being readily coagulated by exposure to air. The proteose, on the contrary, suffers but little, and will endure boiling without precipitation. These alcoholic extracts, according to Goodale, "should be kept as far as possible from exposure to air, and if in the older extracts cloudiness occurs, it should not be filtered, as thereby the albumen would be removed, leaving only the proteose in solution." It is very evident, if alcohol has such an effect upon protein extracts, that some other preservative would be more acceptable, especially when it is desired to use the extracts in an aqueous form.

Several experimenters have obtained satisfactory results with carbolic acid or trikresol, and it would seem that these preservatives would be more satisfactory than alcohol, as they not only will prevent bacterial contamination, but, in the small amount in which they are used, have not a deleterious action upon the proteins themselves. Carbolic acid and trikresol, or alcohol for that matter, are not ideal preservatives, as the extracts in which they are used can not be applied to sensitive surfaces, such as the conjunctiva, on account of their painfully irritating action. To overcome this H. C. Hamilton, of this laboratory, has suggested an oil of high coefficiency which the writer has found best suited for this purpose, as it can be used in a dilution absolutely non-irritating to the most sensitive surfaces and still retain a germicidal action equivalent to either of the other phenols or the alcohol.

As to the methods of preparing extracts for presentation in the most practical form, several suggestions have been offered. The most feasible, up to the present time, depends upon either a direct drying of the aqueous extract or a precipitation of the proteins with alcohol or acetone, followed by a vacuum desiccation of the precipitate. Woodhouse[2] has published a method of preparing desiccated extracts which so far has given favorable results. The aqueous extract is obtained either from cooked (according to method of Goodale) or uncooked foods, and evaporated, if possible, to dryness. The residue is dissolved in sufficient water and precipitated by three or four volumes of 95 per cent alcohol. The precipitate is then centrifuged and washed several times with 95 per cent alcohol, absolute alcohol, and then ether, and finally dried over sulphuric acid. According to Woodhouse, it was necessary to deviate from this general plan by precipitating with a mixture of acetone and ether instead of 95 per cent alcohol. These methods are not entirely satisfactory as the desiccated powder is not always completely soluble in water, and the more the dehydration is delayed, the more insoluble becomes the residue.

Baker and Floyd[3] have also proposed a method of desiccating the protein extracts, especially bacterial extracts, which depends upon keeping the extract at a constant temperature of 40° C. with a water bath while rapid evaporation is produced by means of a current of air from an electric fan directly over the suspension. This well-known method of evaporation is always reliable and can be used in any laboratory, although the material must be well fortified against bacterial contamination. After the extract has been reduced to a powdered form, it is made into a suspension with glycerin in the proportion of 10 mgs. of the powder to 1 c.c. of glycerin. The authors have this to say concerning their mixture: "One or two drops of this preparation are used for a test. If the powder is applied to the skin directly, a constant standard can not be established or a sterile preparation can not be vouched for. The glycerinated preparation insures, with ordinary care, a sterile preparation and a constant strength, thus establishing a standard."

After trying out several of the most promising of the above methods of preparing extracts, the writer has found no one of them entirely satisfactory, the greatest fault being that they take up too much time to apply; this is quite an essential point when patients are to be tested with a large number of extracts.

The following method has been employed by the writer (the clinical application of these extracts to be published later) during the past year, and it has been found most satisfactory, as the extracts are better preserved from deterioration and are in a form more convenient to use than any heretofore described. The protein is first extracted from the fresh or dried material with sterile water containing just enough of the previously mentioned highly coefficient oil to act as a preservative. To this aqueous extract is added enough glycerin so that when the solution is evaporated (any one of the several methods of evaporating may be used) the final amount of extract will be in a quantity sufficient to produce a standard previously decided upon. To this glycerin extract is added a sufficient amount of finely ground boric acid powder in a mortar to make a rather heavy paste. For convenience the author has had this paste put up in the ordinary small collapsible tubes, pressing out just the amount necessary for each test.

In applying the paste, the following simple and convenient procedure was suggested and has since been used to the exclusion of all others. Take upon the end of a sterile wooden toothpick just enough of the paste for a test, rubbing the paste into the scarification with the toothpick, which is then to be discarded.

The advantage of this method of preparing the various protein extracts and presenting them for administration lies in the fact that the crude material is first extracted with water, thus assuring the presence of all available proteins; the extract, which can be accurately standardized, is well preserved from deterioration, especially bacterial contamination, and is in a form suitable for instant use. The paste is perfectly miscible with, and soluble in, the body fluids or in water, is not an irritant in itself, and can be used repeatedly either with or without the collapsible tube with practically no chance of contamination.

If it is preferred to obtain the proteins in a desiccated form, according to the method of Woodhouse and others, they can be applied in the paste form by simply making a heavy suspension of the powder in the glycerin before mixing with the boric acid or by mixing the powder directly with the boric acid.

BIBLIOGRAPHY.

[1] Goodale, J. L.: The Diagnosis and Management of Vasomotor Disturbances of the Upper Air Passages, Boston Med. and Surg. Jour., 1916, clxxv, 181.

[2] Woodhouse, R. P.: Preparation of Vegetable Food Proteins for Anaphylactic Tests, Ibid., 195.

[3] Baker, H. M., and Floyd, C.: Protein Extracts in States of Hypersensitization, Ibid., 199.

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NOTES IN REGARD TO HORSE LICE, TRICHODECTES AND HAEMATOPINUS.*

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Horses are infested with two kinds of lice, the sucking louse, *Hæmatopinus asini (Hæmatopinus macrocephalus)*, and the biting lice, *Trichodectes pilosus* and *Trichodectes parumpilosus*. The two forms are readily distinguishable, as the sucking louse is a larger louse with a long pointed head, and the biting lice are smaller forms with a blunt rounded head. (See figures.) The sucking louse, as the term suggests, is a blood-sucker. The biting louse lives on the superficial epidermal scales and the débris and secretions present on the skin. The sucking louse is probably the more vicious pest of the two, as the loss of blood from the attacks of large numbers of these lice may be a serious matter. There is probably little difference in the degree or amount of annoyance due to the presence and irritation from the two different kinds of lice, but even here the puncturing of the skin by *Hæmatopinus* is perhaps more irritating than the biting of the superficial epidermis by *Trichodectes*. Both forms give rise to itching, and the results from this are often surprisingly unpleasant. A barn full of horses may become a pandemonium as a result of lice. The itching animals attempt to relieve the itching by rubbing and biting, other animals start to kick, presently the kicking becomes general, and there is a resultant clamor and din, with a substantial element of danger to the horses and attendants. Occasionally the lice will give rise to eczema. Lice seem to be more numerous in late winter or early spring. They are more readily detected when horses stand in the warm sunshine, as this brings the lice to the surface. The sucking lice are especially easy to detect on white horses.

Attempts to find exact published information in regard to the bionomics of the horse lice indicate that not very much work has been done along these lines. The large literature on the bionomics of the louse parasitic on man, *Pediculus humanus,* a very large part of which has come into existence since the beginning of the present war, suggests that this lack of information regarding horse lice is not merely apparent, but real. Many persons, writing on horse lice, discuss periods of treatment, remedies, and other matters for biting lice and sucking lice together, apparently on the basis that a louse is a louse and, more especially, that a horse louse is a horse louse. But inasmuch as there is a rather wide gap between a sucking louse and a biting louse, the first belonging to the Hemiptera and the second to the Mallophaga, it might be as safely assumed that there would be considerable difference between the two kinds as regards not only structure, but also bionomics and, consequently, details of treatment. A few very tentative experiments seem to confirm this idea.

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HORSE LICE.

SUCKING LOUSE.

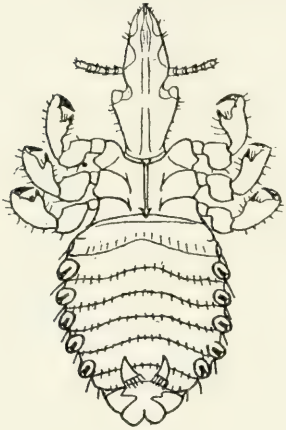

FIG. 1. *Haematopinus asini* (*H. macrocephalus*). Female. ×16. After Neveu-Lemaire (1912).

BITING LICE.

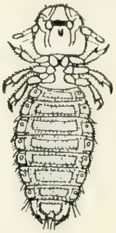

FIG. 2. *Trichodectes parumpilosus.* Female. ×14. After Neveu-Lemaire (1912).

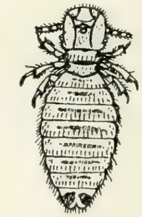

Fig. 3. *Trichodectes pilosus* (*Tr. vestitus*). Female. ×14. After Neveu-Lemaire (1912).

In the purchase of hundreds of horses for the serum work of Parke, Davis & Company, there is more or less trouble as a result of the importation of lice on new purchases, and this problem becomes more acute in winter when dipping is no longer feasible.

During the summer lice may be readily controlled by dipping, a coal-tar dip furnishing a cheap and satisfactory means of freeing horses of lice. During the past winter the writer has assisted the veterinary staff at Parkedale and has undertaken a few rather simple experiments to obtain a little information which did not seem to be available. The principal object of this paper is to make those results available to others and to make public such findings as are in any way new.

In the first place, 38 horses were selected for examination for lice, taking those on which lice had been seen or where the restlessness or evidence of itching suggested the presence of lice. Of these horses, lice were found on 24, or 63 per cent, so that of cases presenting evidences of lousiness clinically, conviction in the shape of lice found was not obtainable in quite a number of cases. Of the 24 horses found infested with lice, 22, or 92 per cent, were infested with sucking lice, *Hæmatopinus asini*, and only 2, or 8 per cent, were infested with biting lice, *Trichodectes pilosus*. The infestations with *Hæmatopinus* ranged from very light to very heavy; those with *Trichodectes* were both very heavy. It is interesting to note that Osborn (1891), contrary to our experience here, found the biting louse most common in Ohio at the time he wrote, and says of the sucking louse, *Hæmatopinus:* "Notwithstanding the probable frequent occurrence of this species, we have as yet failed to meet with examples. The biting lice from horses have been secured in great numbers, but we have searched in vain for this one." Even five years later, Osborn (1896) writes of *Hæmatopinus:* "The biting lice have been secured in great numbers, but we have searched in vain for this one, and but few have come to hand." As regards the species of biting lice found, we are again at variance. I found no specimens of *Trichodectes parumpilosus*, while Osborn (1891, 1896) says of *Tr. pilosus:* "We have not been fortunate enough to secure examples of this form, though we have the other in great abundance." The difference in Osborn's findings and our findings as regards the relative abundance of the two species of biting lice is paralleled in Europe, where Fiebiger (1912) has pointed out that Railliet considers *Tr. pilosus* the more rare, while Taschenberg considers it the more common of the two species. Neveu-Lemaire (1912) agrees with Taschenberg.

Experiments showed a great difference in the length of time the sucking lice could live off their host as compared with the biting lice. In a number of tests, sucking lice, *Hæmatopinus*, lived 1 or 2 days, the last of them never surviving beyond the second day after their removal from the horse. On the other hand, biting lice, *Trichodectes*, lived from 5 to 8 days, the last of them being found dead on the ninth day. One factor which may partly account for this is the fact that both species of lice were kept in vials with small amounts of the horse's hair, removed when the lice were collected. This hair perhaps furnished food for the biting lice, while the sucking lice, deprived of the blood which constitutes their food, speedily starved. In spite of this, the experiment indicates that stables are apt to be capable of infecting horses with biting lice for a period of at least 8 days, at the temperatures noted below, even disregarding the presence of eggs, whereas stables would probably be incapable of infecting horses with biting lice after being kept empty for 2 or 3 days at these temperatures, if we disregard the presence of eggs. The presence of eggs in stables would depend largely on whether the horses involved were shedding or losing hair in other ways. There would seem to be little likelihood of the eggs becoming detached from the hairs. Newly hatched lice of either the biting or sucking kinds died inside of two days when kept off a host animal. I have no data in regard to the humidity conditions of these longevity experiments, and only the temperature range, which was from 21° to 31° C. (70° to 88° F.). The lice would probably live longer at lower temperatures.

Louse eggs may be readily distinguished from the eggs of the bot-flies by the fact that lice attach their eggs to the hairs at a point close to the skin, so that they are not easily seen unless the hair is parted to look for them, whereas the bot-flies attach their eggs on the hairs at a point remote from the skin. Bot-flies commonly attach their eggs to the hairs, and especially the long hair, of the fore-legs, shoulders, nostrils, chin and mouth, places readily accessible to the mouth of the horse, since the larvæ must get to the stomach of the host. In our experience, lice eggs were most common in the flanks and around the angle of the jaw, the latter a point not accessible to the mouth. The eggs of lice and bot-flies can be further differentiated by the fact that the louse egg is a symmetrical affair, the cap of the egg rounding off the unattached end like the lid of a barrel, while the bot-fly egg is asymmetrical, the cap of the egg being set obliquely to the longitudinal axis of the egg. The bot-fly egg is transversely striated, the egg of *Hæmatopinus* is finely stippled, the stippled dots being at the intersections of a network of lines, and the egg of *Trichodectes* is smooth. The eggs of *Hæmatopinus asini* are an opaque creamy white, 1.2 to 1.27 mm. long and 530 to 540 μ thick, measuring mounted specimens and excluding the adhesive mass that attaches the egg to the hair. The eggs of *Trichodectes pilosus* are daintier, more translucent affairs, with an iridescent sheen, and are smaller, 830 to 880 μ long and 390 μ thick. Louse eggs are usually attached to one hair, but an occasional egg is found attached to two hairs.

A few experiments were undertaken to determine the length of time required for the hatching of *Hæmatopinus* and *Trichodectes*. In these experiments, eggs were collected from horses and kept in Petri dishes under atmospheric conditions of humidity and temperature. The humidity factor was not ascertained; the temperature range was the same as that for the longevity experiments, 21° to 31° C. (70° to 88° F.). The length of time the eggs had been on the horse was, of course, unknown, so that the periods obtained only serve to show that eggs may require that much time to hatch; they may require longer.

In the case of *Trichodectes pilosus*, eggs hatched in the course of 5 or 6 days. A majority of the eggs remained unopened at the end of 50 days, the young lice being found dead and shriveled within the shell at the end of that period.

In the case of *Hæmatopinus asini*, eggs hatched in the course of 10 to 18 or 19 days. The large majority of the eggs which were unopened when collected remained unopened at the end of 50 days. These unhatched eggs were dissected open with the following findings: About 66 per cent of the young lice were dead and shriveled; about 13 per cent were dead, but well preserved instead of shriveled; the remainder, about 21 per cent, appeared to have undergone autolysis, the egg content being fluid and unorganized. It appears likely that the failure to hatch on the part of the large majority of the eggs tested is to be attributed to unfavorable incubation conditions rather than to any common tendency to fail to hatch under normal conditions. On the other hand, the examination of large numbers of eggs collected from horses showed a majority of these eggs to be already opened, so that in a given collection of opened and unopened eggs, the unopened ones presumably include those functionally incapable of maturing or opening, which defective eggs would persist on the horse for long periods.

The length of time required for maturation of the lice on horses was not ascertained, and I have been unable to find this point covered by definite experiments in the literature available to me. This knowledge should be available before one can speak with certainty as to the proper intervals for dipping. Since the dips used kill the lice, but not the "nits," we must know the time required for a female which hatches right after dipping to become mature and deposit eggs, for this is approximately the period during which we may postpone dipping. We must not postpone it until the female has actually deposited eggs. The other time factor concerns the length of time required for eggs to hatch. After the first dipping we must wait until the egg deposited just before that dipping has hatched in order to kill all the lice emerging from eggs present at the time of the first dipping. The relations of the hatching period and the period of maturation determine the intervals between dippings and the number of times it is necessary to dip. Since the maturation period for horse lice is not known to me, the procedure indicated for dipping, on the strength of the scanty knowledge available, would be as follows: For sucking lice, if animals are only to be dipped twice, dip at intervals of about 3 weeks; if they may be dipped three times, dip at intervals of about 2 weeks, to eliminate the likelihood of a short maturation period resulting in egg production before the latest of the eggs present at the first dipping had hatched. As a rule, however, the sucking lice mature in 3 or 4 weeks, according to Herms (1915), and practical experience indicates that two dippings are usually ample for lice. On the other hand, the statement by Herms that sucking lice hatch in 5 to 6 days is apparently not true of *H. asini.* The same authority states that biting lice hatch in 5 to 8 days, which accords very well with our findings, and mature in 3 to 4 weeks. Under these conditions, horses should be dipped for biting lice twice at intervals of 10 to 20 days, probably 2 weeks being the safest interval. For 2 dippings, where both kinds of lice are present, an interval of about 20 days appears to be indicated.

The writer appreciates that studies of this sort should be completed and well rounded out before publication if possible. It does not appear possible to complete this study at this time, as this experiment work was only incidental to eradication work, and in view of the shortage of readily available data on this subject it seems advisable to make even this tentative contribution. Moreover, as noted later, the present war calls for all available information along the line of lice control.

As noted earlier in this paper, writers habitually discuss treatments of horse lice as though no distinctions between biting lice and sucking lice need be drawn. I take it that this is due in part to a lack of available detailed information, especially in regard to the bionomics of these insects, on which to make distinctions. The small amount of work that we have done suggests that even in the use of dips, a treatment commonly regarded as equally effective against *Hæmatopinus* or *Trichodectes*, there are differences worth considering, not only in the preferred intervals for dipping, but also in regard to the relative resistance to dips and other insecticides. Tests which are yet incomplete and which will be covered in later papers indicate that *Trichodectes* is more resistant to insecticidal treatment than is *Hæmatopinus*.

A distinction between the effects of treatment on sucking lice and on biting lice of horses which does not appear to have been sufficiently emphasized is the fact that even contact insecticides may be simultaneously "stomach poisons" or ingested insecticides for biting lice. When the horse's skin and its hairy covering is soaked with a dip or covered with a contact insecticide of any sort, it contaminates the food of the biting lice, so to speak, and subjects them to any toxic action arising from ingesting these substances. In this way it may happen that biting lice might survive dilutions of contact insecticides that would kill sucking lice, and yet fall victim to these contact insecticides as a result of their actual action as stomach poisons. As matters stand, we would not recognize this action and would regard the death of the biting lice, which might follow the dipping rather closely, as the result of the insecticidal action by contact. This matter deserves investigation.

Along this same line, the writer would like to record the result of tests of an ingested insecticide on horse lice. In the Annual Report of the Chief of the United States Bureau of Entomology, issued in 1916, Dr. L. O. Howard notes that the work done in that bureau has demonstrated that all the species of lice infesting poultry may be readily destroyed by the application of a very small quantity of sodium fluoride. Sodium flouride is one of the common stomach poisons used in combating such insects as roaches, and is the base of many roach powders. The method of using sodium fluoride for chickens is given by Bishopp and Wood (1917) as follows: Hold the bird by the legs or wings and put a pinch of sodium fluoride on the head, one on the neck, two on the back, one on the breast, one below the vent, one on the tail, and one scattered on the underside of each wing when spread; or dip the bird in tepid water containing ¾ to 1 ounce of commercial sodium fluoride, or ⅔ of an ounce of the chemically pure material, to each gallon of water.

There does not appear to be any reason why sodium fluoride should not be as effective for the biting lice of mammals, species of *Trichodectes,* as for the lice of birds, all of which are biting lice belonging to various genera of *Mallophaga.* In order to test this, however, one of the two horses infested with biting lice was treated with sodium fluoride, the powder being taken in handfuls and rubbed into the hair practically all over the body. The other horse was treated with a contact insecticide. Both horses were very lousy before treatment and both were completely freed of lice by their treatments. A number of other horses infested with sucking lice were also treated with sodium fluoride, while others were treated with various contact insecticides. The sodium fluoride treatment, as was expected, was not successful in destroying sucking lice. While a single experiment with one horse would not of itself establish the efficacy of a treatment, the fact that this success is in accord with the other known facts in regard to biting lice and their treatment makes a rather strong case for this treatment, and without caring to say that its value is established beyond question, we have no hesitancy in saying that it is probably as effective as the experiment indicates and is worthy of trial.

The sodium fluoride treatment has the advantage of being applicable in winter, and it apparently does not injure the hair or skin. It has the disadvantage that it is only applicable to biting lice and that a horse covered with powder is not very attractive or even useful in some ways for the time being.

Methods for the control of lice are of special interest at this time. The bringing together of large numbers of horses from all sorts of places in war times habitually results in a rapid spread of these pests and great annoyance to the horses. This, in time, results in trouble for the cavalry and field artillery and work for the army veterinarian. And right now many of us must regard ourselves as potentially army veterinarians. If the present war makes the demands on this country which already seem possible, there will probably be a need for veterinarians in and out of the army that will require some stretching of our abilities to fill. In view of the importance which the subject of lice control may assume in the near future, I venture to summarize here a few of the control measures which may prove useful.

Obviously, the first control measure is directed against the lice present with a view to killing them. Lice are not especially difficult to kill. The coal-tar dips are cheap and effective, and are the things most commonly used in this country. The Prussian army uses the following: Infusion of tobacco (1 to 25 or 30), with or without the addition of vinegar, which treatment has the disadvantage of being poisonous; sabadilla vinegar, also poisonous, and to be used well rubbed in at infested spots only; fish oil, which may be used in the same way; 1 part of petroleum to 10 of methylated spirit; equal parts of petroleum and rape-seed oil; 2 to 3 per cent watery solution of creolin or 3 per cent solution of liquor cresolis compound; grey mercury ointment, not over 150 grains at one application, rubbed over the entire body, either alone or mixed with oil or soft soap, taking care to keep it out of the eyes; or, in case of need, washing the horse with soapy water, and while the coat is still damp, dusting on finely sifted beech ashes or peat ashes and brushing in well. Inasmuch as soap is a good insecticide of itself, it is likely that some relief could be obtained by the use of strong soap-suds if nothing better was available. Louse powders, containing oxytoluol, crude cresol, naphthalin, pyrethrum, sulphur and other more or less insecticidal substances, may be used. They have the advantage of being available in winter weather when dips cannot be safely used as a rule; they have the disadvantage noted in the discussion of sodium fluoride of temporarily detracting from a horse's attractiveness and usefulness and are not such effective and simple contact insecticides as dips. The sodium fluoride treatment, as already noted, may be used against biting lice.

Aside from killing the lice present, there are certain prophylactive and adjuvant measures of value. Clipping horses is a great aid in lice control. Plenty of brushing and currying is another aid. Dipping the curry comb and brush in kerosene or a strong coal-tar dip when leaving one horse and passing to another is a measure of value in preventing the spread of lice; due regard must be had for the potential danger of kerosene as a source of fire loss in a stable, and for the fact that kerosene is depilatory for horses. Also, too strong coal-tar preparations irritate the skin. Shake off the excess kerosene or coal-tar preparation after dipping the comb or brush. Where horses can be curried on a picket line, it diminishes the danger of reinfection from lice and eggs which are cleaned off, since these are more apt to perish out of doors and not return to their hosts than would be the case with lice in a stable. It also diminishes the fire risk in using kerosene. The danger of transmitting lice on saddle blankets, horse blankets, and bridles has not been well investigated or reported; the danger exists but it is uncertain whether it is considerable or negligible. To be on the safe side, these things should be regarded as carriers of infestation and treated accordingly. Cleanliness is almost always valuable in the control of parasites. Clean stables, frequently whitewashed or washed down with preparations of germicidal and insecticidal character, are safer than dirty stables. The prompt and frequent removal of manure and stable litter is a measure of value. Segregation of badly infested horses and quarantine measures for new animals are measures that are indicated when feasible.

It is interesting to note that a number of European investigators have stated that one way to remove the human body louse from clothing is to put the clothing on a horse. Some writers advance the theory that there is something insecticidal about this treatment and about horses. One of these writers makes the following statements: Lice are rare on horses; baker's assistants protect themselves against fleas by putting their sleeping clothes with the coats of the stable hands; the Ruthenian people put their clothing over horses to get rid of lice; dogs roll in horse manure to get rid of fleas. Another writer surmises that the efficacy of the system of putting the clothing on horses is not due to a repellent odor, as stated by some, but to the lice abandoning the clothing to get on the horse for warmth and nutrition, and then perhaps being killed by the impalpable and irritant powder from the skin. These surmises are interesting without being entirely convincing. Having seen thousands of lice swarming over horses, it is hard for us to believe that a horse is an insecticide. Still it is conceivable that the human louse (Nuttall states that there is no dependable morphological difference between the head louse and the body or clothes louse), if transplanted to a horse, might die of something akin to gastralgia or even nostalgia.

In conclusion, I would like to acknowledge the courteous assistance and co-operation of Dr. R. H. Wilson and Dr. L. A. Maze in the conduct of the experiments noted here.

BIBLIOGRAPHY.

Bishopp, F. C., and Wood, H. P. 1917. Mites and lice on poultry. Farm. Bull. No. 801, Wash., 26 pp., 14 figs.

Fiebiger, Josef. 1912. Die tierischen Parasiten der Haus- und Nutztiere. Vienna and Leipzig, 428 pp., 302 figs.

Herms, Wm. B. Medical and Veterinary Entomology, N. Y., 393 pp., 228 figs.

Neveu-Lemaire, Maurice. Parasitologie des animaux domestiques. Maladies parasitaires non bacteriennes. Paris, 1257 pp., 770 figs.

Osborn, Herbert. 1891. The Pediculi and Mallophaga affecting man and the lower animals. Div. Entom. Bull. No. 7, Wash., 56 pp., 42 figs.

1896. Insects affecting domestic animals: An account of the species of importance in North America, with mention of related forms occurring on other animals. Div. Entom. Bull. 5, n. s., Wash., 302 pp., 170 figs.

103812

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"THE SUSCEPTIBILITY OF THE PRAIRIE DOG TO RABIES."*

GEORGE WALTERS.

(From the Research Laboratory of Parke, Davis & Co., Detroit, Mich.)

The significance of this paper does not at once present itself even to those most familiar with the prairie dog as a constant neighbor. In fact, it was not apparent to the writer until three of these animals were offered to the laboratory as substitutes for rabbits. Their vicious temperament and apparent intractability led us to suspect that they would make good subjects for experimental rabies. They were therefore set aside for observation and treatment.

Having but three animals it was decided to keep one for control and attempt, if possible, to establish a certain degree of immunity in the others so that some check could be had upon the action of the virus to which the animals were to be ultimately exposed. It was also believed that the course of the disease could be observed more intelligently by proceeding in this manner.

The following gives the result of this experiment in detail:

Animal No. 1; *Weight,* 1350 grams; *Antirabic Vaccine,* Special P. D. & Co. Experimental product; *Exposed,* 21 days after last injection of vaccine. Intracranial injection of 0.2 c.c. fresh virus; *Paralysis,* 13th day; *Result:* Died 16th day of rabies. Animal extremely nervous and very vicious during treatment. Following inoculation irritability increased, would attack dogs and had to be kept in closed cage.

Animal No. 2; *Weight,* 1140 grams; *Antirabic Vaccine,* Special P. D. & Co. Experimental product; *Exposed,* 21 days after last injection of vaccine. Intracranial injection of 0.2 c.c. fresh virus; *Result:* Immune to date (5-18-17). Nearly three months after death of other animals.

Animal No. 3; *Weight,* 1075 grams; *Antirabic Vaccine* (control); *Exposed,* 21 days after last injection of vaccine. Intracranial injection of 0.2 c.c. fresh virus; *Paralysis,* 10th day slight paralysis right leg. 11th day coming down; *Result:* Died 13th day following inoculation.

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It will be seen from the foregoing table that a fairly typical rabies infection may be suspected. The subsequent inoculations from the brain of these animals gave corroborative results.

It was not intended that this report of rabies in the prairie dog should be considered as final. Neither was it intended to be particularly of a rabies immunity problem. There are too many technical considerations to be allowed for such a problem on so few animals, as unrecognized protein reactions, meningeal symptoms from other than rabic causes simulating rabies, and a total lack of extensive experiments on these animals with which the present series may be compared.

A thorough search of the literature fails to show any previous record of rabies infection among prairie dogs. Nor are we able to ascertain whether this branch of the marmot family has been studied with any special reference to health problems. It is well known that other species may be easily infected, and marmots in Asia and the western slope of the Rockies have been studied with reference to the plague problem.

Prior to 1914 when Henry W. Henshaw, Chief of the Biological Survey of the U. S. Government, reported his studies of the marmot, the relationship and ranges of many of the species of this rodent were quite imperfectly known.

Economic reasons were the cause of various investigations. In 1900 the Bureau of Animal Industry furnished an extensive review of losses endured by cattle men of the Southwest on account of the destructive effect of the prairie dog upon their grazing lands by their extensive colonization. The review was followed by many important articles during the succeeding twelve years, but no hint of the health problem was mentioned. This seems almost incredible in view of the well known temperamental morbidity of these animals with their "nasty" tempers. In spite of its social exclusiveness and apparent timidity, so well known, the animal shows a marked disposition toward viciousness.

The prairie dog or prairie marmot (Cynomys), of which the best known species is *Cynomys ludovicianus,* is closely related to the squirrel, chipmunk, rat, cavidæ, porcupine, hare, gopher, chinchilla, wood-chuck, and other species of rodents.

Of this order it shows anatomically marked resemblance to all the various species and physiologically identical characteristics.

They are essentially a "social" animal living in immense colonies ranging from a few acres to thousands of square miles in area, the population averaging from 35 to 64 per acre. They are not easily captured, and when taken they remain long savage and intractable. They may be tamed, however. Efforts to tame the animals, reported in this paper, failed during a period of several years and no laboratory experiments could be undertaken with them because of their consistent ugliness.

Their natural enemies are the coyotte, badger, black-footed ferret, and a few denizens of the arid regions, among which may be named especially the owl and the rattlesnake, both of which feed upon the young and receïve no mercy from their very harmless-looking captors, who bury them in their holes when possible.

The prevalence of rabies among coyotes has recently been the subject of serious legislation and health department control in the west, and the facts in the foregoing paragraph indicate how easily rabies may be spread among prairie dogs by these or other enemies, and thence to animals which might come in contact with them offensively. When it is borne in mind that their instincts lead them to almost heroic attempts at self-preservation when attacked or angered, this becomes doubly apparent.

It is hoped this paper will lead to further studies by others on the marmot family as related to rabies. Certain problems closely related to it will be the subject of later reports from this laboratory.

BIBLIOGRAPHY.

Henshaw, H. W. Chief Biol. Survey; Report on Relationships and Species of Marmots, 1914.

Howell, Arthur H. U. S. Dept. Agriculture Bul. No. 37, 1915.

Merriam, C. Hart. Year Book U. S. Dept. Agriculture, Washington, D. C., 1901. Also Marmots of Adirondacks, N. Y., 1882.

Peters, A. F., and Avery, S. Report on Destruction of Pests and Certain Legislation. Washington, D. C., 1901.

Hoare, E. W. System Vet. Med., Vol. 1, 1913.

Müller, G., and Glass, A. Diseases of Dog and Treatment, 1908.

Sime, D. Rabies.

Farnebaugh, Capt. E. Amer. Jour. Pub. Health, 1916.

Moodelly, P. S. Ind. Annals. Med. de Calcutta, 1859—VI—201.

Hutyra and Marek. Path. and Ther. of Diseases of Domestic Animals.

Yount, C. E. The Coyote and Rabies. So. Cal. Pract., Mar., 1910.

U. S. P. H. Reports.

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MANURE DISPOSAL AS A FACTOR IN THE CONTROL OF PARASITIC DISEASES OF LIVE STOCK.

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The results of the work on hookworm disease of man in the southern United States and elsewhere in the world has emphasized the major importance of sanitation and especially of a proper disposal of feces as a control measure for this disease. Not only hookworms, but the other worms parasitic in the digestive tract and its adnexa in man, depend on the feces first of all as a vehicle for their transmission. But this is equally true for the parasitic worms infesting our live stock and domesticated animals. The eggs or larvae of stomach worms, nodular worms, ascarids, *Strongyloides,* whipworms, tapeworms, flukes and even lungworms pass first to the outer world in the manure, and a proper disposal of the manure is the first line of prophylactic attack.

While the importance of a proper disposal of feces has been emphasized and is being emphasized as a control measure for such parasites of man as the hookworm, the proper disposal of manure as a control measure for a large number of important parasites of live stock has not been adequately emphasized. Yet the fact is that this point deserves special consideration. Our live stock is constantly exposed and re-exposed to parasitic infestation by virtue of the fact that these animals eat off the ground, so to speak, to a great extent. Our horses, cattle, sheep and goats graze on pasures, our hogs root up the ground in search of white grubs and hunt through swampy pig-pens for the corn which is dumped into the mud, our chickens pick most of their food off the ground or out of the soil, and even our dogs, as a rule, seem to think as much of food that has been tossed out in the back yard as of food presented in a clean utensil. This mode of feeding exposes the food of these animals to contamination, since the feces of the animals are deposited on the ground, and the area in which the animal habitually spends its time is the area in which it feeds and which it contaminates. The prevention of this condition is for the most part impossible. The prevention of parasitic infestation as a result of this condition is a matter that calls for repeated treatment with suitable and adequate anthelmintics, thereby cutting down the supply of worms in the animals and so shutting down egg production at its source. Vigorous measures along this line may be expected to render the manure reasonably safe on the score of parasitic infestation, since parasite eggs must be produced in large numbers in order to permit of perpetuation of parasites under the operation of the laws of chance.

But while the manure deposited on the pasture is not a thing that can be readily controlled, the manure from the barns and other buildings where domesticated animals are housed is something that can be more or less controlled. Contrary to the general rule for human feces, barn-yard manure must be utilized as fertilizer, which means that it must go back to the fields somewhere. Now if the animals furnishing this manure were never on pasture and were always fed from clean troughs and racks, they would tend to lose any parasitic infestation they might have, the manure simultaneously tending to become free from parasite eggs and larvæ and safe for use on the fields anywhere so far as parasites were concerned. I have seen old horses which had been used in a large city and kept off pasture which had almost none of the strongyles of the large intestine which are so common in horses as a rule, and sheep raised under experiment conditions which were almost free or completely free from stomach worms. But the farm animal from which barnyard manure or stable manure is obtained is usually only a transient in the barn or barnyard or stable. These animals are in the pasture every day or a part of the year, and the barn-yard or stable manure is usually as infective to begin with as is that on the pasture.

There are two obvious lines of attack in disposing of the infective features of this manure. One would consist in treating the manure in such a way as to kill the parasite eggs and larvæ, and the other would consist in disregarding this feature and placing the manure where it would have little or no capacity for infecting stock.

The first method offers a field for a large amount of investigation, but this investigating has not been done. Broadly speaking, one would have to determine how long the eggs and larvæ of the various species of worms involved lived in manure piles, in spread manure, in closely packed manure; the effect of sunlight; the effect of moisture; the effect of various chemicals, the chemicals in turn being of a nature not to injure the fertilizer value of the manure. There are practically no data on this and little could be surmised without such data.

The second method is one concerning which we have adequate data. It merely involves the application of facts which are fairly well known, but which have received little emphasis in this connection. We have emphasized to a certain extent the value of stock rotation on pasture as a control measure for parasites. We know that if a pasture has one kind of stock on it year after year it becomes highly infested with the eggs and larvæ of the parasites which infest that kind of stock. We might state this briefly and alliteratively as follows: *Permanent pastures perpetuate parasites.* Such pastures are dangerous—for that kind of stock. They may be quite harmless for other kinds of stock. Thus horses may safely follow swine or follow ruminants (cattle, sheep and goats); swine may follow horses or ruminants; ruminants may follow horses or swine. These groups have worm parasites which in general are not transmissible to each other, so that the manure from one group is not infective for the other groups. The fact that all the ruminants are infested with the deadly stomach worm makes it undesirable to attempt to rotate cattle, sheep and goats with one another.

But the extension of this idea of stock rotation to the disposal of barn-yard manure and other manure has not been emphasized. I do not remember ever seeing the idea stated, yet it is probably in print. At any rate it deserves to be emphasized. Manure which is to be taken to the fields should be disposed of, wherever possible, with a view to its possibilities as a carrier of parasitic infestation. It will not always be possible to make the application. There are dairy farms where only cattle are kept, and the manure from these cattle must go back to the pastures and fields where the cattle will graze, unless exchange is made with neighboring farms running different kinds of stock. There are horse farms, hog farms, and sheep farms in a somewhat similar situation. In such cases one might bear in mind that well rotted manure would be safer than manure which had not been held long or rotted. The time element involved here permits of the death of eggs and larvæ as a result of various unfavorable conditions, and the rotting process may well prove lethal, since in nature the worm eggs are washed onto the pasture and the larvæ are capable of traveling, in some instances, yards away from the manure deposits. It is also likely that lime would kill larvæ when added to manure.

However, there are farms where there is an option as to the disposal of the manure of two or more kinds of stock. Horse manure may be put where horses are to run or where cattle are to run; where such alternative offers, it is advisable to put it where cattle are to run. The same principle applies to manure of ruminants, swine and horses. Where there is a considerable amount of poultry droppings, as there sometimes is, it may be regarded as safe for anything other than birds, and manure from other animals, generally speaking, will convey no parasites to birds. On the other hand, the feces of man and the dog are always to be regarded as dangerous. While it is true that there is little necessity for considering the disposal of quantities of dog feces, it is also true that the casual straying over our farms by dogs maintains a persistent supply of gid in sheep, hydatids in swine, other bladderworms in sheep and cattle, and other parasitic pests. While it is true that we do not make a practice of fertilizing our soil with human feces in this country as they do in China, it is nevertheless true that the informal practice exists in this country even if there is no statement of it as a formal agricultural measure. It is well known that in the Southern States there has been, and still is over a large territory, an almost total neglect of sanitary provision for disposal of feces in the rural districts, and something of this neglect is true for some parts of the Northern rural districts. In human feces we not only have the source of numerous parasites of man, but the source of such parasites of our stock as "measles" (cysticercosis) in cattle and swine.

It may be urged that much of this barn-yard manure is plowed under and the fields so treated sowed to crops which will be gathered and not fed back to stock. Quite true; and that it is a safe method of avoiding infestation. On the other hand, the plowing under of manure for forage crops cannot be regarded as assuring safety. There are possibilities for the survival of eggs and larvæ and the likelihood that larvæ would escape from the manure and soil under certain conditions.

What we are urging is no panacea, and it is not of universal applicability. We only urge that manure be regarded as the most important means of conveying parasitic infestation; that it be regarded as potentially dangerous; that in situations where there is an option in disposing of manure, it be placed where animals of the sort furnishing the manure will not be exposed to parasitic infestation from it. This same measure will also serve as an aid in the control of some bacterial diseases.

Studies from the Research Laboratory
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THE RATIONALE OF POLLEN EXTRACT THERAPEUSIS OF HAY FEVER, WITH A REPORT OF 127 CASES.

By Newell S. Ferry, M.D., Detroit, Mich.
(From the Research Laboratory, Parke, Davis & Co.)

The theory has been advanced and is generally accepted that hay fever is a symptom complex following a toxemia depending upon a parenteral digestion of proteins of certain pollen.

To Holbrook Curtis[1] has been given the credit of first endeavoring to produce an active resistance against the disease, although all writers have recognized the pioneer scientific work of Dunbar,[2] not only as regards the etiological relationship of pollen to the disease, but also as to his endeavors to establish the value of a specific antiserum. Unfortunately Dunbar's experiments were based on his erroneous conception of the similarity of pollen extract, the so-called pollen "toxin," to a true toxin. As a result, he was led into the error of attempting to prove that an antitoxin could be obtained which, *in vivo* as well as *in vitro*, would neutralize the pollen extract in a manner similar to the action of diphtheria antitoxin toward diphtheria toxin.

It has been conclusively shown that Dunbar's pollen extract is not a toxin, according to the generally accepted meaning of the term, as it reveals none of the characteristics essential to a true toxin. It is poisonous only to a small part of the human species and apparently innocuous to the animal species, except in large doses. It is thermostable, is not affected by acids or alkalies, and the intoxication has no incubation period, nor does it follow the law of multiple proportions.

Recently Cooke, Flood and Coca[3] have stated that "Hay fever is the clinical symptomatic expression of local hypersensitiveness. The active pollen substances are not toxins."

As the extract is not a true toxin it is impossible to conceive of Dunbar's antiserum ("Pollantin") as being a true antitoxin, although in extremely large doses it might exhibit some neutralizing effect.

While Curtis, in 1900, reported favorable results against hay fever with extracts of the whole plants and flowers, it was not until 1911 that the first scientific work was carried out along lines of so-called active immunity. At that time Noon[4] and Freeman,[5 6] from the laboratory of the Department for Therapeutic Inoculation, St. Mary's Hospital, London, described their results with aqueous extracts of the pollen of grasses. The extracts were prepared after a method, first used by Dunbar for obtaining his pollen toxin, which consisted of the addition of distilled water to a certain amount of pollen, the extraction being aided by repeated freezing and thawing. Koessler[7 8] at the same time was, in this country, working along somewhat similar lines with favorable results, but his findings were not published until 1914.

Prior to the work of Noon and Freeman, Dunbar had demonstrated that the application of a "barely invisible trace" of pollen to the conjunctival or nasal mucous membrane almost instantly produced most intense symptoms of hay fever in subjects sensitive to that particular pollen. The same experiment was negative to those not suffering with the disease. It was later shown also that aqueous extracts of the same pollen gave similar reactions. By this means was established a method of differentiating between those individuals sensitive to one pollen from those sensitive to another, and of determining equally well the exact resistance of a patient toward and given pollen extract. This diagnostic method proved conclusively that hay fever depended upon an individual predisposition, and this, together with the fact that pollen extracts have been considered of a protein nature, led many to believe that anaphylaxis was the underlying cause of the disease. The conception that hay fever was a symptom of anaphylaxis and resulted from the parenteral digestion of the proteins of pollen was, however, first suggested by Wolff-Eisner.[11]

The specific local reactions, manifested in hay fever sensitive individuals, following the ophthalmic, cutaneous, or intracutaneous applications of pollen extracts, seem to show that at times the blood and tissues contain a proteolytic ferment which is capable of splitting off the poisonous group from the pollen proteins. As a result of this cleavage the poisonous group, acting as an irritant, stimulates the local inflammatory conditions, which are recognized as the specific reactions. If these local reactions, and the general reaction known as hay fever, are the result of anaphylaxis the patient must necessarily have previously been sensitized. The method or methods bringing about this primary hypersensitization have, however, never been satisfactorily explained. According to Koessler "* * * the individual disposition to hay fever may have been inherited or may have been acquired during any period of life." Cooke and Vander Veer[9] conclude that "sensitized individuals transmit, to their offspring, not their own specific sensitization, but an unusual capacity for developing bioplastic reactivities to any foreign proteins."

In opening the way for the initial parenteral absorption of the protein which produces the acquired sensitization, something must have occurred which resulted in an interference with the normal digestive function of the nasal mucosa, as it is generally believed that all mucous mmbranes will readily digest protein material under normal conditions with no untoward effects. Of the many explanations that have been forthcoming as regards the predisposing cause of the primary sensitization, perhaps the following are the most plausible: an insufficient nasal secretion concomitant with a lowered percentage of normal proteolytic enzymes; or an excessive accumulation of pollen, either accidentally inhaled or resulting from some abnormal anatomical obstruction. While the term "active immunity," originally used in this connection by Noon, is still a popular expression for this type of an increased tolerance, yet it is a question whether it is correct, and it will probably not be decided until the status of the protein molecule has been fixed and its relation to antigens has been determined. In a "Study of the Chemistry of Pollen Extracts" the author and Mr. Lewis Davis[10] of this laboratory have found that the active principle appears to be of the nature of deuteroproteose.

PROPHYLAXIS.

Noon and Freeman, accepting the work of Dunbar, believed that the pollen extract contained a toxin and, on that basis, proposed a method of treatment aiming at the production of an active immunity. The same method is practically carried out today, although the rationale of the treatment is founded upon an entirely different premise. At the present time it is believed that hay fever results from protein sensitization and that a sensitized individual may be desensitized by compelling the body cells to exhaust all of the available specific proteolytic enzymes upon pollen protein artificially injected. Cooke, Flood, and Coca tell us that the mechanism of the alleviating effect of specific therapy is the same as that of desensitization in experimental anaphylaxis. In other words, a condition of anti-anaphylaxis is produced rendering the patient practically refractory or immune, so that when the protein reaches the mucous membrane, through the agency of the pollen in the natural way, it is not rapidly split up but is allowed to liberate the toxic part slowly, as under normal conditions, and the symptoms universally recognized as hay fever do not appear.

While several methods of desensitizing an individual against a foreign protein have been suggested, the most promising, as regards the pollen protein, seems to be by the subcutaneous route, starting with extremely minute doses of the protein, as represented by the pollen extract, and gradually raising the tolerance by increasing the size of the dose.

In order to determine a safe initial dose of the pollen extract, or a dose that will not produce a severe reaction, a very ingenious method, based upon the local reaction, as first brought out by Dunbar, was proposed by Noon. It was found that an individual, when sensitive to a certain pollen, always gave an ophthalmic reaction with an aqueous extract of that particular pollen and, by decreasing the strength of the testing material, a condition was reached when no reaction was discernible. This was considered the limit of the resisting power of the tissues of the patient against the extract, and by this means the exact status of the tolerance of the patient was determined or, in other words, the exact amount of extract, which could with safety be injected into the patient, was estimated. In this country the cutaneous or intracutaneous methods of diagnosis have apparently supplanted the ophthalmic, as they not only give a more accurate index of the patient's resistance, but also permit of several tests being carried on at the same time, to be repeated daily if necessary.

With these means at hand for making a correct diagnosis and of estimating the resistance of the patient toward any pollen extract there is practically no element of danger in the treatment, provided single extracts are used. Multipollen extracts or extracts of pollen to which the patient is not sensitive should not be injected subcutaneously until more is known of the subject of protein sensitization and the exact relation of the protein molecule to hay fever has been determined. There is an element of danger in sensitizing the individual, during the course of the treatment, to a protein of a pollen which the patient, up to that time, was able to digest and absorb with safety. And, for this reason, a patient should not be injected with pollen extract before the diagnosis has been determined, and the specific relation has been established between the extract in question and the pollen to which the patient is sensitive.

POLLEN.

While it has been shown by Scheppegrell,[12 13 14 15] President of the American Hay-Fever Prevention Association, Goodale[20 21] of Boston and others, that a large number of pollens will produce hay fever, and that the disease may be found at any time of the year, yet, with the great majority of cases, in this country, there are but two distinct seasons corresponding to the pollenizing of the two classes of plants, the Graminaceæ of the spring and the compositæ of the fall.

Scheppegrell[16 17 18 19] has given us four groups which cover nearly all the hay fever cases; the Ambrosiaceæ, Graminaceæ, Artemisiæ, and Chenopodiaciæ.

Treatment with pollen extracts is also much simplified by the fact, in a large percentage of cases, that sensitization to one member of a plant family or group may be diagnosed by using extracts of the pollen of other members of the same family or group, making it necessary to determine only the member giving the strongest or the most typical reaction. This has been corroborated, in whole or in part, by Freeman, Goodale, Koessler, Cooke and Vander Veer, and Scheppegrell.

As a result of a large amount of experimental and clinical work the past few years, the most important members of the various groups have been shown to be as follows: ragweed pollen of the Ambrosiaceæ, timothy pollen of the Graminaceæ, mugwort pollen of Artemisiæ, and the chenopodium pollen of the Chenopodiaceæ.

The popular notions relative to rose colds and golden-rod fever have practically been ruled out, as the majority of observers maintain that only wind-borne pollen are to be considered in hay fever.

EXTRACTION OF POLLEN.

Dunbar extracted the pollen with distilled water or salt solution, aided by freezing and thawing.

Noon's method was practically the same as Dunbar's with the exception that Noon sterilized his extract by boiling in sealed glass tubes.

Koessler first thoroughly broke up the dried pollen in an agate mortar and then extracted with 8.5 per cent saline solution. This extract was diluted with ten times its volume of distilled water to make a .85 per cent solution of salt. He used 0.25 per cent phenol as a preservative. He also prepared a desiccated extract by precipitating his saline extract with ten times its volume of 95 per cent alcohol, the precipitate being filtered and dried. Koessler claims that his saline extract deteriorates very rapidly, producing, by progressive proteolysis, a toxic product within 8 to 10 days. He maintains that his concentrated extract can be kept on ice for three weeks.

Goodale makes a 12 to 15 per cent alcoholic extract. He also makes the statement that his extract will deteriorate within a short time, especially if exposed to the air.

Sormoni[23] extracted first with distilled water, after which he made a physiologic salt solution and added carbolic acid as a preservative.

Oppenheimer and Gottlieb[24] [25] extracted the pollen with 5 per cent sodium chloride in 0.5 per cent carbolic acid solution after being ground in mortar with sand. The mixture was then placed in a thermostat for 72 hours, after which it was precipitated with eight volumes of absolute alcohol and filtered quickly. The aqueous extract, according to these authors, deteriorated on standing.

Lowdermilk[29] first ground the pollen in a damp state with 0.5 per cent carbolic acid solution. It was then diluted and stock solution allowed to stand in a cool place.

Clews[27] first removed the oily membrane from the pollen with acetone before extracting.

Cooke[22] extracted with 0.8 per cent sodium chloride in n/100 NaOH in a mortar with sand, precipitated with three volumes of acetone and redissolved in 0.8 per cent sodium chloride, and then filtered through a Berkefeld filter. His later work was done with an extract which was not precipitated.

The author, after trying several of the preceding methods, found that the simplest was the most practical. He ground the pollen in a ball mill with 0.85 per cent saline solution in 0.5 per cent high coefficient phenol. This mixture was diluted and shaken for 24 hours, after which it was filtered through a Berkefeld candle and then standardized. The final extract, unlike those of Koessler, Goodale and others, will not readily deteriorate. It has been found potent in a dilution of 1 to 100,000 in room temperature over a year after its preparation, and after two years when kept at refrigerator temperature. It is the author's opinion that an aqueous extract, if properly prepared, will remain stable under ordinary conditions for at least two years.

STANDARDIZATION OF EXTRACTS.

There are practically but two methods of standardizing pollen extracts; one based on Noon's method of diluting an extract made from a certain amount of pollen and the other by determining the total nitrogen in a given amount of extract and diluting accordingly. In both cases the extracts are compared with an arbitrarily determined unit.

Noon's method, which is the simplest, was followed by the author. Noon chose, as an arbitrary unit, that quantity of pollen toxin which can be extracted from the thousandth part of a milligram of pollen. In other words, one gram of pollen contains one million units of pollen toxin (active principle or protein). With this extract he determined the dilution which just gave an ophthalmic reaction in a susceptible individual, and regulated the doses accordingly. Until more is known of protein sensitization and its relation to the action of pollen extracts, this seems to be the most rational and the safest method of controlling the administration of the extracts, as it would be impossible to give any but a safe dose. Even if every lot of pollen extract were standardized according to the nitrogen content, the resistance of the patient should be determined, as with Noon's extract, for all sensitized individuals are not resistant to the same degree.

In order to simplify the method of administering the extracts as much as possible the author made three dilutions as follows:

Dilution No. 1, 1-100,000 or 10 units per c.c.; No. 2, 1-10,000 or 100 units per c.c.; No. 3, 1-1000 or 1000 units per c.c. With these dilutions any amount of extract may be injected from one unit or less up to 1,000 units, and by using a graduated syringe the amounts will range from .1 c.c. to 1 c.c., it not being necessary to inject more than 1 c.c. at one time. Even with an ordinary syringe the calculations can be made with enough accuracy to warrant the injections being given with safety.

REPORT OF CASES.

At this time the author wishes to report the results of the prophylactic treatment of 127 cases, 9 treated with timothy pollen extract and 118 with ragweed pollen extract.

All but two of these cases were diagnosed with the intracutaneous test; 100 unit solution being used. The remaining cases were diagnosed with the cutaneous test; 1000 unit solution being used.

Of these 127 cases, 86 (68 per cent) weathered the pollen season with practically no symptoms. These cases were tabulated as *relieved*. Twenty-two cases (17 per cent) were more or less relieved, although at times they were greatly annoyed with symptoms of hay fever; tabulated as *benefited*. Nineteen cases (15 per cent) were not relieved—in fact two cases were made worse by the treatment.

RESULTS OF PREVIOUS INVESTIGATORS.

It is impossible to compare the results of previous workers, as all cases were not tabulated the same, and all extracts were not prepared under similar conditions. The conclusions, therefore, can only be tentative, but at least they give an idea of the general trend of results:

Some authors, in their tabulations, use the terms "cured," "greatly relieved," and "not relieved." As it is impossible to say whether a case is cured or relieved for the season only, the author has tabulated all cases under three headings: relieved, benefited and negative. All cases tabulated as cured and greatly relieved under the heading relieved, and all cases more or less relieved under the heading benefited.

The following tabulations are of the cases treated with pollen extract reported in the literature to date, with a comparison of the present series:

PROPHYLACTIC TREATMENT.

Author	Cases, number	Relieved, percent.	Benefited, percent.	Negative, percent.
Sormoni	14	85.7	....	14.3
Others reported by Sormoni	19	100	....	....
Lowdermilk	3	100	....	....
Manning[29]	21	66.6	10	14.4
Oppenheimer and Gottlieb	84	83.3	....	16.7
Ellern[31]	13	46.2	38.5	15.3
Clowes	8	100	....	....
Howe[33]	16	62.5	....	37.5
Strouse and Frank[32]	16	25	37.5	37.5
Cooke and Vander Veer	450	71	18.5	10.5
Total	644	74.3	11.3	14.7
Present series	127	67.7	17.3	15
Total prophylactic	771	70.9	14.3	14.8

PHYLACTIC TREATMENT.

Author	Cases, number	Relieved, percent.	Benefited, percent.	Negative, percent.
Oppenheimer and Gottlieb	15	53.3	....	46.7
Sormoni	12	33.3	25	41.7
Others reported by Sormoni	3	100	....	....
Lowdermilk	16	81.2	....	18.8
Ulrich[39]	11	90.9	....	9.1
Strouse and Frank	5	60	40	....
Howe	26	42.3	57.7	....
Lovell[28]	5	100	....	....
Cooke and Vander Veer	209	69.2	23.1	7.7
Total	302	70.0	16.2	13.8

TYPE OF TREATMENT NOT SUFFICIENTLY DESIGNATED.

Author	Cases, number	Relieved, percent.	Benefited, percent.	Negative, percent.
Noon and Freeman	84	64.3	26.2	9.5
Koessler	41	80.5	12.2	7.3
Total	125	72.4	19.2	8.4

TOTAL OF ALL CASES.

Type of Treatment	Cases, number	Relieved, percent.	Benefited, percent.	Negative, percent.
Prophylactic	771	70.9	14.3	14.8
Phylactic	302	70.0	16.2	13.8
Type of treatment not designated	125	72.4	19.2	8.4
Total	1,198	71.1	16.5	12.4

NEGATIVE CASES.

Of the negative cases there are manifestly three separate classes or types: those that are not desensitized, those that are made worse by the treatment, and those that are desensitized to one pollen while remaining sensitive to some other pollen or pollens.

No doubt many of those in the first class would finally reach a state of complete anti-anaphylaxis if larger doses of the extracts were given. This method would theoretically call forth and use up the remaining specific proteolytic enzymes. A few individuals are found who will stand and apparently demand larger doses of the proteins before a proper neutralization of their ferments is obtained. For patients of this type the doses should be increased and treatment should continue even after the pollen season has begun and symptoms of hay fever made their appearance.

On the other hand it is hard to explain why some patients are made worse by the treatment. As a rule this type of a case will stand the treatment up to about 200 to 300 units and then symptoms of hay fever will appear after each treatment, as severe and at times worse than the disease as found during the pollen season. Attempts have been made to desensitize these patients by repeating the treatment, starting again with small doses and gradually increasing them, but they have failed. When the dose reaches a certain size, symptoms will again appear. It is a question whether favorable results would not follow if the proteins were introduced by some other route.

In order to relieve those of the third type it would be necessary to determine the individual pollen producing the symptoms and treat accordingly.

REMARKS.

1. The 1-1000 solution (1000 units) should be used for the cutaneous diagnostic test.

2. The 1-10,000 solution (100 units) should be used for the intracutaneous diagnostic test.

3. It is not necessary at first to test the resistance of the patient, as the large majority react with the 1-100,000 solution (10 units). It is safe to start all cases with two units and increase

the dose as rapidly as possible, depending upon the general reaction of the patient to the extract; injections being given about every two or three days. If symptoms of hay fever make their appearance as a result of the injections, the dose is too large, and the subsequent doses should be smaller.

4. Experience has taught that the average individual requires from ten to fifteen injections not exceeding 1000 units for the final dose—to produce the resistance necessary to insure against attack. In other words, it takes about 3000 units, or the extract from three milligrams of pollen, to completely desensitize a patient.

5. For phylactic treatment sufficient figures are at present not available. Some cases have been aborted after two or three injections, while others have received over ten injections before any signs of improvement have appeared.

6. Prophylactic treatment where possible is preferable to phylactic.

7. The question naturally arises, how long does the apparent immunity or the desensitization last? Freeman[5] the only writer who has attempted to answer this question gives the following: "It will be seen that where a patient has been inoculated for one year with complete success, he has in the next year complete, or almost complete, immunity, but that in the third year he has only slight immunity left. Where patients have been successfully inoculated for two years, they have, as might be expected, complete immunity during the third year, and time will show how long this complete immunity will last. When patients have been only partially immunized for one or two years, little, if any, immunity is carried over to the year during which they have ceased the inoculations." As these deductions are based on only twenty cases, it would seem necessary to await further reports before drawing any definite conclusions. From a theoretical standpoint, Cooke, Flood, and Coca have answered as follows: "The freedom from symptoms lasts as long as the respective 'antigenic' substances remain in combination with the antibody-like substances."

8. Railroad or automobile trips, through sections of the country where pollen abounds, are extremely liable to break down the acquired resistance of those who would otherwise be free from the disease under the ordinary conditions of every-day life. Ex-

treme exposures should be avoided as much as possible as desensitization is not always absolute.

9. From the present knowledge of protein sensitization, multipollen extracts or extracts of pollen of different groups or families to be used in a "gunshot" sense are not indicated.

CONCLUSIONS.

When single extracts are used and proper precautions taken, pollen extracts give us one of the most, if not the most, scientific methods of prophylactic treatment known to medicine. The reasons are these: the diagnosis can be made, the exact dose calculated, and the resistance of the patient determined before, during and after the period of treatment.

Seventy-one per cent of cases are relieved of practically all symptoms, and at least 16 per cent more are more or less benefited.

BIBLIOGRAPHY.

1 Curtis (New York Med. News, 1900, LXXVII, p. 16).
2 Dunbar (Jour. Hyg., 1913, XIII, p. 105).
3 Cooke, Flood and Coca (Jour. of Immun., 1916, II, p. 217).
4 Noon (The Lancet, 1911, I, p. 1,572).
5 Freeman (ibid., 1911, II, p. 814).
6 Freeman (ibid., 1914, I. p. 1,178).
7 Koessler (Illinois Med. Jour., Springfield, 1914, XXVI, p. 120).
8 Koessler (Forchheimer's Therapeusis of Internal Diseases).
9 Cooke and Vander Veer (Jour. of Immun., 1916, I, p. 201).
10 Ferry and Davis (to be published).
11 Wolf-Eisner: After Schettenheim and Weichhardt (Deutsche med. Wchnschr., 1911, XXVII, p. 867).
12 Scheppegrell (Jour. Am. Med. Assn., 1916, LXVI, p. 707).
13 Scheppegrell (ibid., 1916, LXVII, p. 861).
14 Scheppegrell (U. S. Pub. Health Reports, 1916, III, p. 129).
15 Scheppegrell (New York Med. Jour., 1916, CIV, p. 1,127).
16 Scheppegrell (Am. Jour. Pub. Health, 1917, VII, p. 173).
17 Scheppegrell (Interstate Med. Jour., 1917, XXIV, p. 488).
18 Scheppegrell (Boston Med. and Surg. Jour., 1917, CLXXVII, p. 42).
19 Scheppegrell (U. S. Pub. Health Reports, 1917, XXXII, p. 29).
20 Goodale (Boston Med. and Surg. Jour., 1915, CLXXIII, p. 42).
21 Goodale (ibid., 1916, LXVII, p. 861).
22 Cooke (The Laryngoscope, 1915, XXV, p. 108).
23 Sormoni (The Lancet, 1916, I, p. 348).
24 Oppenheimer and Gottlieb (New York Med. Jour., 1915, CI, p. 229).
25 Oppenheimer and Gottlieb (Med. Record, 1916, LXXXIX, p. 508).
26 Lowdermilk (Jour. Am. Med. Assn., 1914, LXIII, p. 141).
27 Clews (Proc. Soc. Exper. Biol. and Med., 1913, X, p. 70).
28 Lovell (The Lancet, 1912, II, p. 1,716).
29 Manning (Jour. Am. Med. Assn., 1915, LXIV, p. 655).
30 Ulrich (ibid., 1914, LXII, p. 1,220).
31 Ellern (Deutsch. Med. Wchnschr., 1912, XXXVIII, p. 1,590).
32 Strouse and Frank (Jour. Am. Med. Assn., 1916, LXVI, p. 712).
33 Howe (Long Island Med. Jour., 1916, X, p. 188).

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THE INFLUENCE OF THE METHOD OF ADMINISTRATION UPON THE DEGREE OF TOXICITY OF STROPHANTHUS PREPARATIONS.

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The use of strophanthus in therapeutics as a heart tonic is becoming more wide-spread from year to year. Digitalis has for a long time been the favorite cardiac remedy and still maintains a high degree of usefulness, but its slow and more cumulative action contrasted with the more rapid and more certain effect of an active principle such as strophanthin is largely the reason for the increased use of strophanthus preparations. There is another factor which has greatly influenced the use of heart tonics in general and of strophanthus in particular, and that is the increasing use of our only means of determining the activity of these preparations, namely, physiological standardization. The extreme potency and variability of strophanthus preparations have long been recognized in an approximate sense, but it was only as recently as 1897 and 1898, when Houghton[1] introduced the first satisfactory quantitative method of physiologic assay for heart tonics, that our knowledge of the comparative potency and variability of strophanthus preparations became definite. Since that time the standardization of heart tonics by physiologic methods has been slowly taken up and perfected, so that by its aid the manufacture of comparatively uniform strophanthin and tincture strophanthus has been accomplished.

As the use of strophanthus increased the clinical data established certain therapeutic doses of each preparation for oral administration. Authorities differed somewhat as to the average oral dose of the most active preparations, ouabain and strophanthin, largely, as they thought, because of variable absorption, but since preparations are not so extremely potent by oral administration no great harm was done other than causing some confusion.

The greatest trouble and confusion was experienced, however, when clinicians began to follow the general trend of recent medication and to administer strophanthin hypodermically or intravenously. The pharmacology of strophanthin had not been very carefully studied or emphasized, though Hatcher and Bailey in 1908[2, 3] called attention in a rather general way to the increased toxicity of strophanthin when administered hypodermically. This warning was apparently not heeded, as the physician in most cases has followed the generally accepted rule of using about half the recognized oral dose for each hypodermic or intravenous administration.

The serious difficulties encountered in hypodermic usage demand that further attention be drawn to the somewhat peculiar action of strophanthus preparations in order to emphasize and thereby minimize the danger attending their hypodermic or intravenous administration. It is with this purpose in mind, as well as with a desire to add to our scientific knowledge of the comparative activity of such important drugs, that I have carried out a series of experiments which may be briefly outlined as follows:

Four strophanthus derivatives which have been or which might be used for hypodermic administration were selected, namely, Kombe Strophanthin, Ouabain (Gratus Strophanthin), Tr. Strophanthus U.S.P. 9th Rev., and Strophanthone Dilute. The degree of toxicity of each was carefully determined by three different methods of administration, namely, oral, subcutaneous and intravenous. The animal used throughout the experiments was the guinea-pig.

The sample of Kombe Strophanthin (the strophanthin from pharmacopœial drug) used was one of Merck's products, which had been assayed by the M.L.D. frog method and found to be of average activity (100,000 H. T. U. per gramme based on an M.L.D. to frogs of .0000010 grm.).

The sample of ouabain used was a Merck product which had previously been found to be the most active of three samples examined by the one-hour frog method, but which contained 185200 H. T. U. per gramme when tested by the M.L.D. method. It was consequently not quite twice as active as the Kombe Strophanthin by the latter method.

Tincture of Strophanthus, though not adapted to hypodermic or intravenous injection in the undiluted form, is however so active that it can be diluted to such a degree if occasion should arise, that it could be injected hypodermically without serious results. It was therefore thought advisable to determine the intravenous and subcutaneous M.L.D.'s of this preparation in order to make the comparisons more complete. The average tincture has 1300 H. T. U per mil.

Strophanthone Dilute was the fourth and last member of the series. This is an aqueous solution of the active ingredient of strophanthus and consequently is well adapted to hypodermic and intravenous administration. It is standardized by physiologic assay and contains 100 H. T. U. per mil in ampoules.

The following tables give the results of toxicity tests of the four preparations upon guinea-pigs, three different methods of administration being used:

TABLE I.

Kombe Strophanthin—Oral Administration.

Pig No.	Wt., gms.	Dose in gm. per gm.	Dil.	Total dose.	Result.
1	296	.000015	1-100	.44	Lived
2	328	.000020		.66	Lived
3	290	.000025		.73	Lived
4	255	.000025		.64	Lived
5	247	.000030		.74	Lived
6	240	.000035		.84	Died
7	265	.000030		.80	Lived
8	262	.000030		.79	Lived
9	247	.000035		.87	Lived
10	247	.000035		.87	Lived
11	316	.000035		1.11	Lived
12	341	.000040		1.36	Died
13	291	.000040		1.16	Lived
14	354	.000040		1.42	Lived
15	327	.000040		1.31	Lived
16	317	.000045		1.43	Died
17	291	.000045		1.31	Died
18	301	.000045		1.35	Died

M.L.D. of Kombe Strophanthin orally is .000045 gramme per gramme body weight.

TABLE II.

Ouabain (Gratus Strophanthin)—Oral Administration.

Pig No.	Wt., gms.	Dose in gm. per gm.	Dil.	Total dose.	Result.
1	339	.000005	1-500	.85	Lived
2	394	.000015		2.95	Died
3	300	.000010	1-100	.30	Lived
2	316	.000010		.32	Lived
5	323	.000010		.32	Lived
6	339	.000012		.41	Lived
7	296	.000015		.45	Lived
8	286	.000015		.43	Lived
9	263	.000015		.39	Lived
10	336	.000020		.67	Died
11	254	.000020		.51	Died
12	248	.000020		.50	Died

M.L.D. of Ouabain orally is .000020 gramme per gramme body weight.

TABLE III.

Tincture Strophanthus U. S. P. -Oral Administration.

Pig No.	Wt., gms.	Dose in Cc. per gm.	Dil.	Total dose, Cc.	Result.
1	454	.0008	1-3	1.09	Lived
2	465	.0010		1.40	Lived
3	463	.0012		1.67	Lived
4	457	.0015		2.06	Lived
5	428	.0018		2.31	Lived
6	500	.0018	1-2	1.80	Lived
7	554	.0020		2.22	Died
8	584	.0020		2.34	Died
9	566	.0020	None	1.13	Died
10	518	.0020	1-2	2.07	Died
11	540	.0022		2.38	Died
12	382	.0022		1.68	Died
13	430	.0025		2.15	Died
14	475	.0025		2.38	Died
15	381	.0025		1.91	Lived
16	362	.0025		1.81	Died
17	441	.0030		2.65	Died

M.L.D. of Tr. Stroph. orally is .0020 mil per gramme body weight.

TABLE IV.

Strophanthone Dilute—Oral Administration.

Pig No.	Wt., gms.	Dose in Cc. per gm.	Dil.	Total dose,mils.	Result.
1	225	.09	None	20.25	Died
2	252	.08		20.00	Died
3	280	.07		19.60	Died
4	293	.06		17.58	Died
5	212	.05		10.60	Died
6	257	.04		10.28	Died
7	281	.03		8.43	Lived
8	260	.04		10.40	Lived
9	287	.04		11.48	Lived
10	303	.03		9.09	Lived
11	327	.03		9.81	Lived
12	378	.02		7.56	Lived
13	358	.05		17.90	Lived
14	320	.05		16.00	Lived
15	330	.06		19.80	Died
16	230	.06		13.80	Died

M.L.D. of Strophanthone Dilute orally is .060 mil per gramme body weight.

TABLE V.

Kombe Strophanthin—Subcutaneous Administration.

Pig No.	Wt., gms.	Dose in gm. per gm.	Dil.	Total dose.	Result.
1	393	.0000008	1-6000	1.45	Died
2	248	.0000009		1.34	Died
3	316	.0000007		1.33	Died
4	320	.0000006		1.15	Died
5	340	.0000005		1.02	Died
6	461	.0000004	1-8000	1.48	Lived
7	412	.0000005		1.65	Died
8	384	.0000006		1.84	Died
9	298	.0000004	1-5000	.60	Lived
10	297	.00000045		.67	Died
11	291	.00000045		.65	Died

M.L.D. of Kombe Strophanthin subcutaneously is .00000045 gramme per gramme body weight.

TABLE VI.

Ouabain (Gratus Strophanthin) Subcutaneous Administration.

Pig No.	Wt., gms.	Dose in gm. per gm.	Dil.	Total dose.	Result.
1	452	.00000020	1-10,000	.90	Lived
2	406	.00000025		1.02	Lived
3	375	.00000030		1.13	Died
4	423	.00000025		1.06	Died
5	355	.00000030		1.07	Died
6	321	.00000020		.64	Lived
7	355	.00000015		.53	Lived
8	325	.00000025		.81	Lived
9	314	.00000025		.79	Died
10	278	.00000025		.69	Lived

M.L.D. of Ouabain subcutaneously is .00000030 gramme per gramme body weight.

TABLE VII.

Tr. Strophanthus U. S. P. Subcutaneous Administration.

Pig No.	Wt., gms.	Dose in Cc. per gm.	Dil.	Total dose.	Result.
1	256	.00003	1-50	.38	Lived
2	331	.00005		.83	Died
3	311	.00007		1.09	Died
4	363	.00003		.54	Lived
5	347	.00004		.69	Lived
6	346	.00005		.87	Died
7	367	.000045		.83	Died
8	350	.000045		.83	Died
9	382	.000040		.78	Died
10	355	.000040		.71	Died
11	310	.000040		.62	Lived
12	336	.000035		.59	Lived
13	302	.000040		.60	Lived

M.L.D. of Tr. Strophanthus subcutaneously is .000045 mil per gramme body weight.

TABLE VIII.

Strophanthone Dilute Subcutaneously Administered.

Pig No.	Wt., gms.	Dose in Cc. per gm.	Dil.	Total dose.	Result.
1	280	.0004	1-5	.56	Lived
2	233	.0006		.70	Died
3	226	.0008		.90	Died
4	367	.0004		.73	Lived
5	353	.0005		.88	Lived
6	320	.0006		.96	Lived
7	352	.0006		1.06	Died
8	347	.0007		1.22	Died
9	345	.0007		1.21	Died
10	323	.0005		.81	Lived
11	309	.0007		.93	Died

M.L.D. of Strophanthone Dilute subcutaneously is .00060 mil per gramme body weight.

TABLE IX.

Kombe Strophanthin—Intravenous Administration.

Pig No.	Wt., gms.	Dose in gm. per gm.	Dil.	Total dose.	Result.
1	384	.0000005	1-4000	.77	Died
2	485	.0000002	1-5000	.49	Lived
3	475	.0000003		.71	Lived
4	385	.0000004		.72	Died
5	493	.0000003		.74	Lived
6	540	.00000035		.95	Died
7	232	.0000003		.80	Died
8	340	.0000003		.82	Lived
9	558	.00000025		.68	Lived
10	312	.00000025		.87	Died

M.L.D. of Kombe Strophanthin intravenously is .00000035 gramme per gramme body weight.

TABLE X.

Ouabain (Gratus Strophanthin)—Intravenous Administration.

Pig No.	Wt., gms.	Dose in gm. per gm.	Dil.	Total dose.	Result.
1	376	.00000020	1-10,000	.75	**Died**
2	342	.00000010		.34	**Lived**
3	354	.00000015		.53	**Died**
4	428	.00000012		.51	**Lived**
5	463	.00000015		.69	**Lived**
6	360	.00000020		.72	**Lived**
7	407	.00000015		.61	**Lived**
8	353	.00000020		.71	**Died**
9	322	.00000020		.65	**Died**
10	335	.00000015		.50	**Lived**

M.L.D. of Ouabain intravenously is .00000020 gramme per gramme body weight.

TABLE XI.

Tr. Strophanthus U. S. P.—Intravenously Administered.

Pig No.	Wt., gms.	Dose in Cc. per gm.	Dil.	Total dose.	Result.
1	544	.000020	1-50	.54	**Lived**
2	582	.000040		1.16	**Died**
3	361	.000050		.90	**Died**
4	493	.000030		.74	**Died**
5	476	.000025		.59	**Died**
6	582	.000020		.58	**Died**
7	447	.000020		.45	**Lived**
8	530	.000015		.40	**Lived**
9	577	.000020		.58	**Lived**
10	463	.000025		.58	**Died**

M.L.D. of Tr. Strophanthus intravenously is .000025 mil per gramme body weight.

TABLE XII.

Strophanthone Dilute—Intravenously Administered.

Pig No.	Wt., gms.	Dose in Cc. per gm.	Dil.	Total dose.	Result.
1	550	.0003	1-5	.83	**Lived**
2	413	.0005		1.03	**Died**
3	357	.0004		.81	**Died**
4	360	.00045		.81	**Died**
5	337	.00050		.84	**Died**
6	375	.00045		.84	**Died**
7	355	.00040		.71	**Lived**
8	375	.00045		.84	**Died**

M.L.D. of Strophanthone Dilute intravenously is .00045 mil per gramme body weight.

TABLE XIII.

Summarized Results—Minimum Lethal Doses.

Preparation.	Orally.	Subcutaneously.	**Intravenously.**
1. Kombe Strophanthin	.000045 gm. per gm.	.00000045 gm. per gm.	**.00000035 gm. per gm.**
2. Ouabain	000020 gm. per gm.	.00000030 gm. per gm.	**.00000020 gm. per gm.**
3. Tr. Strophanthus U.S.P.	.0020 mil per gm.	.000045 mil per gm.	**.000025 mil per gm.**
4. Strophanthone Dilute	.060 mil per gm.	.00060 mil per gm.	**.00045 mil per gm.**

TABLE XIV.

Ratio of M.L.D.'s of the same preparation by different methods of administration.

Preparation.	Subcu. to oral.	Intravenous to oral.	**Intravenous to subcu.**
1. Kombe Strophanthin.	100 times.	128.6 times.	**1.28 times.**
2. Ouabain.	66.6 times.	100.0 times.	**1.50 times.**
3. Tr. Strophanthus.	44.4 times.	80.0 times.	**1.80 times.**
4. Strophanthone Dil.	100.0 times.	133.0 times.	**1.33 times.**

TABLE XV.

Toxicity ratios of different preparations by the same methods of administration.

Preparation.	Orally.	Subcu.	Intravenously.
K. Strophanthin to Ouabain.	0.44 times.	0.66 times.	0.57 times.
K. Strophanthin to Tr. Stroph.	44.4 times.	100.0 times.	711.4 times.
K. Strophanthin to Strophanthone Dil.	1333.3 times.	1333.3 times.	1285.7 times.
Ouabain to Tr. Stroph.	100.0 times.	150.0 times.	125.0 times.
Ouabain to Strophanthone Dil.	3000.0 times.	2000.0 times.	2250.0 times.
Tr. Stroph. to Strophanthone Dil.	30.0 times.	13.3 times.	18.0 times.

TABLE XVI.

Toxicity ratios for the same preparations, upon different animals.

Preparations.	Guinea-pigs, subcu.	Frog, subcu.
K. Strophanthin to Ouabain.	0.66 times.	0.5 times.
K. Strophanthin to Tr. Stroph.	100.0 times.	77.0 times.
K. Strophanthin to Strophanthone Dil.	1333.3 times.	1000.0 times.
Ouabain to Tr. Stroph.	150.0 times.	153.8 times.
Ouabain to Strophanthone Dil.	2000.0 times.	2000.0 times.
Tr. Stroph. to Strophanthone Dil.	13.3 times.	13.0 times.

The first twelve tables give a detailed account of each toxicity test and need no further comment. In the last four tables the results obtained have been summarized in different ways. A careful consideration of these tables serves to bring out several important and unusual facts.

In the first place, in Table XIV the great differences in degrees of toxicity of the same preparation when administered hypodermically and when given orally is clearly brought out. Thus Kombe Strophanthin and Strophanthone are 100 times as toxic when given subcutaneously as when given orally, and even Tincture of Strophanthus, which shows the least difference, is 44 times as toxic by the subcutaneous method as by oral administration. The increased toxicity by intravenous administration over subcutaneous dosage is very much less, being in no case over 1.8 times as toxic for the same preparation. It is thus apparent from this table of relative toxicities that the satisfactory therapeutic dose of any of these preparations for intravenous or subcutaneous use cannot be derived from the previously determined therapeutic dose for oral administration by the usual rule of giving ½ to ¼ as much. With the ratios of toxicity subcutaneously to orally ranging from 100 to 44 times for the different preparations, serious results would be almost certain to follow the subcutaneous administration of one-half the therapeutic oral dose.

Hatcher[4] first observed the marked difference in toxicity of strophanthin when given subcutaneously and orally. He accounted for this fact partly by saying that the excretion more nearly kept pace with the slow absorption following administration into the alimentary canal. He also advanced the theory that strophanthin may be partly destroyed in the alimentary canal. The view held by Cushny[5] that because of the glucosidal nature of strophanthin the abnormally low oral toxicity is *largely* due to destruction of the strophanthin in the alimentary canal by the ferments of the digestive tract, is a very logical and satisfactory explanation of the peculiar action of strophanthus. If this be true, the oral administration of strophanthus preparations is illogical and certain to cause confusing results, since the destruction and absorption will not be uniform. The absorption following hypodermic administration is much more rapid and certain, since there is slight opportunity for the destruction of the active principle. The greatest caution must be observed, however, particularly in the *intravenous* use of strophanthus preparations, that the dose is not too large.

Strophanthin for hypodermic or intravenous use is usually placed on the market in 1-mil ampoules of a 1-to-1000 solution. The activity of such a solution of the average Kombe Strophanthin, according to tests upon frogs, is just equivalent to that of Strophanthone Dilute in 1-mil ampoules. For intravenous administration .5 mil of such solutions should be large enough to produce the desired therapeutic effect.

Ouabain or Gratus Strophanthin has been recently recommended for hypodermic or intravenous use because of its purity and supposed uniformity. Recently published tests[6] show a lack of uniformity in samples of ouabain, but the *average* activity is about twice that of Kombe Strophanthin (a statement first made by Gley[7]), and consequently it should be borne in mind that only half as large a dose should be given.

Table XV serves to show just how much more toxic Kombe Strophanthin and Ouabain are than Tincture of Strophanthus and Strophanthone Dilute by three methods of administration to guinea-pigs. It also shows how closely the relation is maintained in the different methods.

Table XVI gives a comparison of the toxicities of the different preparations as determined upon frogs and guinea-pigs. The comparatively close agreement is rather remarkable.

CONCLUSIONS.

1. The subcutaneous and intravenous toxicities of the four strophanthus preparations tested are from 45 to 100 times as great as their oral toxicities.

2. It has been demonstrated that the satisfactory oral dose is not a true index of the potency of strophanthus.

3. To obtain the most uniform and satisfactory therapeutic results strophanthus preparations should be administered hypodermically. Extreme caution should however be exercised in selecting a sufficiently small dose for subcutaneous and intravenous injection.

BIBLIOGRAPHY.

1. Houghton: *Jour. Amer. Med. Assoc.*, 1897, xxix, 517; 1898, xxxi, 959.
2. Hatcher and Bailey: *Jour. A. M. A.*, 1909, lii, 5-10.
3. Hatcher: *Am. Jour. Phys.*, 1909, xxiii, 303.
4. Hatcher and Bailey: *Jour. Amer. Med. Assoc.*, 1910, lv, 1697.
5. Cushny: Pharmacology and Therapeutics, Sixth Edition.
6. Rowe: *Jour. Amer. Pharm. Assoc.*, 1916, v. 1183.
7. Gley: *Comptes Rendus*, cvii, 348.

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Studies from the Research Laboratory
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COCCOBACILLUS (FOETIDUS) OZAENAE OF PEREZ.*

PLATES 1 AND 2

Herbert C. Ward

(From the Research Laboratory of Parke, Davis & Co., Detroit, Mich.)

The coccobacillus of Perez is of practical interest because of its supposed rôle in the ozena stage of atrophic rhinitis. It has been isolated from ozena by Horowitz, Perez, Hofer, and in this country by Horn and Ward.[1] So far the reports have not dealt extensively with the character of the organism, and in this article are given the principal results of a study of several strains both from European sources and obtained here from patients.

Nasal exudates are inoculated in broth tubes and incubated for 15-20 hours, when subcultures are made on other media. This is a good method because the Perez bacillus practically outgrows the other organisms introduced into the broth. The following statements are based on the detailed study of 12 strains selected from 50 isolated altogether:

The bacillus is small, varies in diameter, the ratio of breadth to length being 1:1-1:4. Some of the strains assume distinctly bacillary forms on all media, while others are often coccoid. Coccoid forms occur especially in liquid medium and in mass growths; on potato atypical forms are frequent. The bacillus forms no chains, no spores, is gram-negative, but otherwise stains readily. The European strains are non-motile, and this is the case also with strains isolated here, although some appear to have become motile on cultivation, while others seemingly have been sluggishly motile from the first.

The organism is easily cultivated. Agar colonies are moist, round, translucent, bluish pale, about 2 mm. in size, the center after a few days assuming a yellow-brown tinge. On agar slants luxuriant growth develops, moist, translucent, with wavy borders, faint yellow-brown streaks appearing in older cultures, without odor. Agar stabs give a good growth, without pigmentation as a rule, the growth at the end of the puncture being slight. After 5 days at 37° C., gelatin stabs develop a uniform turbidity without liquefaction. On blood serum the growth is moist, glistening, faintly yellowish, and without odor. On potato there develops a moderate, moist, gray or faintly yellow growth, without odor. Broth becomes turbid in a few hours; in old cultures a delicate blue film forms on the surface and an unpleasant nauseating odor develops, different from that produced by B. proteus, B. coli, and the cocci. A small amount of acid is produced in litmus milk without clotting after 7 days. Dextrin, levulose, lactose, saccharose, maltose, glycerol, inulin, and mannite are not fermented; glucose alone is reduced, but fresh cultures may not act until 3-5 days; after 10 days the amount of gas produced may run 15-75%; the ratio of CO_2:H :: 2:3. In broth to which litmus, methylene blue, or neutral red have been added, there is complete reduction in 3-4 days.

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[1] Jour. Infect. Dis., 1916, 19, p. 153. For other references see this article.

In deep gelatin colonies pigment develops, so that after 24-72 hours the center of the colonies appears, by transmitted light, as a mahogany-brown, lobulated mass within a lighter brown zone, and after 4-5 weeks at room temperature the colonies may appear as black dots on a white background. Occasionally old broth cultures develop a color so that they look like turbid claret.

The action of the bacillus on protein material is characteristic. In Dunham medium peptones are broken up and indol, skatol, ammonia, hydrogen sulphid, methylmercaptan, and other by-products are formed. The same sort of decomposition occurs also in broth, the process being only partly interfered with by carbohydrates.

Undoubtedly the odor in ozena and the odor of broth cultures of Perez' bacillus is due to volatile sulphur compounds.

In rabbits the intravenous injection of culture filtrates may cause death in 48 hours, and the injection of killed and washed bacilli may give rise to ulceration. No evidence of the formation of a true bacterial toxin has been obtained. The intravenous injection of living cultures in rabbits produces, as shown by Perez and others, increased secretion from the nose, a thin watery discharge developing, which later becomes mucopurulent. It is possible to establish in this way a chronic condition, with crusts about the nostrils. The bacillus is easily recovered from the nose, and may be recovered from the blood, spleen, and other organs. When large doses are given, death may occur within a short time; after smaller doses, death may occur after several weeks, preceded by emaciation. Some rabbits are immune.

After subcutaneous injections, at 3-7 day intervals of increasing quantities of culture 500, heated for 10 minutes at 60° C., agglutinins were developed that clumped European and American strains in dilutions of the serum of 1:2000. All the strains mentioned in Table 1 were agglutinated by the same immune rabbit serum diluted 1:1000.

TABLE 1.

INTRAVENOUS INJECTIONS OF PEREZ' BACILLUS.

Strains	Dose, C.c.	Nasal and General Reactions	Result
500	2.5	Marked nasal secretion	Died in 24 hours
500	2.0	Nasal crusts, emaciation	Died in 42 days
500	2.0	Nasal crusts, emaciation	Died in 32 days
500	2.0	Nasal crusts, emaciation	Died in 17 days
12.2	3.0	Moderate secretions	Died in 15 hours
12.2	2.0	Moderate secretions	Died in 5 days
12.2	1.5	Slight secretions	Recovery
12.2	1.0	Slight secretions	Recovery
23.6	2.0	Moderate secretions	Died in 57 days
23.6	1.0	Moderate secretions	Died in 3 months
23.6	1.0	Slight secretions	Recovery
23.6	0.7	Slight secretions	Recovery
11.3	3.0	Marked secretions	Died in 18 hours
11.3	2.0	Marked secretions	Died in 12 hours
11.3	2.0	Marked secretions	Died in 24 hours
11.3	1.0	Slight secretions	Recovery
29.1	2.5	Marked secretions	Recovery
29.1	2.0	Slight secretions	Recovery
29.1	1.0	Slight secretions	Recovery
29.1	7	Marked secretions	Died in 48 hours
71.4	7	Slight secretions	Died in 24 hours
8.2	1.0	Slight secretions	Recovery
8.3	1.0	Slight secretions	Recovery
500H	1.0	Slight secretions	Died in 4 days

The characteristics of the bacillus of Perez may be summarized as follows: It is gram-negative, grows well in mixed broth cultures, ferments glucose, decomposes proteins with the rapid formation of mercaptan, produces a characteristic pigment in gelatin, and evokes specific agglutinins in rabbits. These characteristics are adequate to distinguish it from other bacilli, with which it may be associated, such as B. pyocyaneus, B. coli, B. mucosus, B. proteus, and B. bronchisepticus. The bacillus is readily cultivated from the exudate in ozena, with which condition it is closely associated.

EXPLANATION OF PLATES

PLATE 1

FIGS. 1, 5. European strains, 24-hour agar growths. ×1200.

FIGS. 2, 3, 4. American strains, 24-hour agar growths. ×1200.

PLATE 2

FIG. 6. Pigment in gelatin culture after 1 month at room temperature, American strain.

FIG. 7. Pigment in gelatin culture after 1 month at room temperature, Vienna strain.

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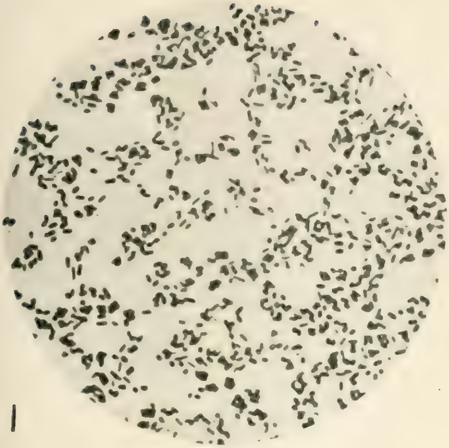
PLATE I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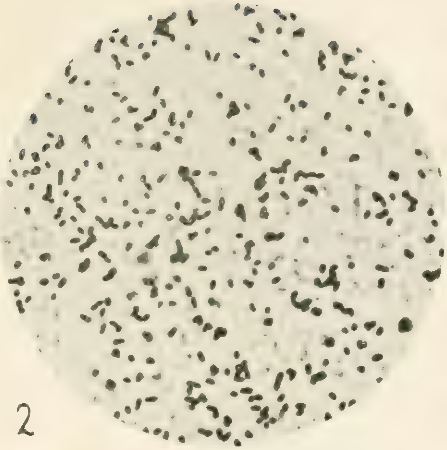
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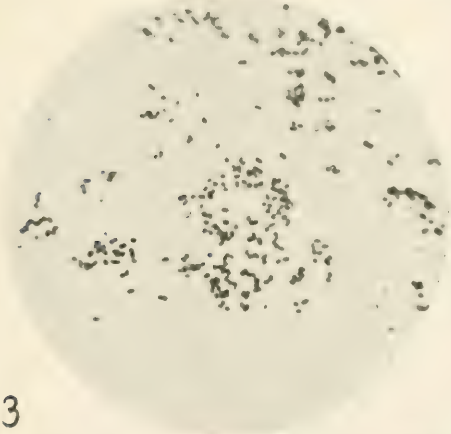
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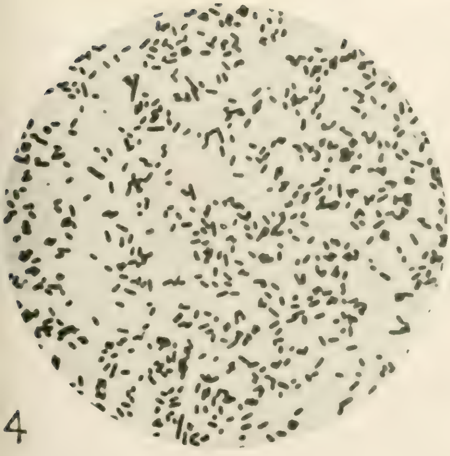
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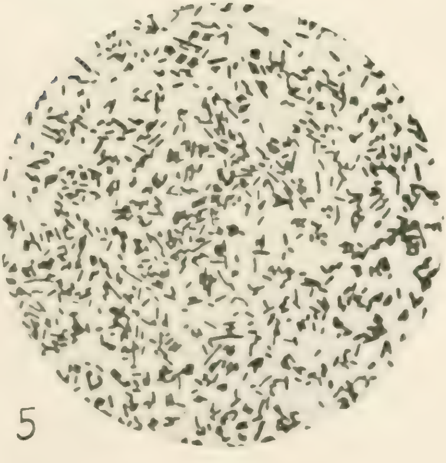
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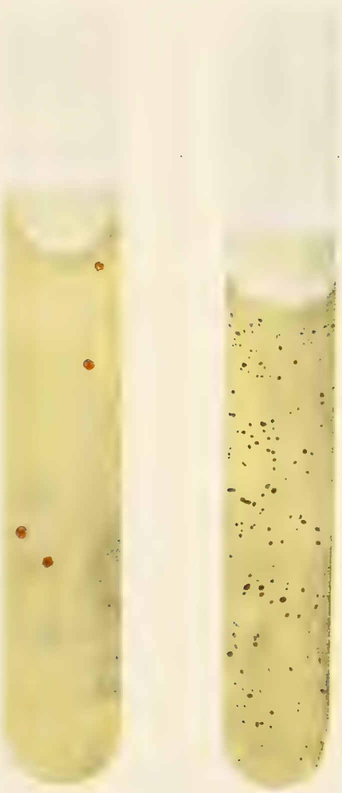
PLATE 2.



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NOTES IN REGARD TO BOTS, *GASTROPHILUS SPP.*

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In connection with some anthelmintic investigations, the writer has taken occasion recently to test the efficacy of carbon bisulphide in expelling bots from horses. Carbon bisulphide has been used for this purpose for some years and has been generally satisfactory, so far as clinical observation shows anything; it removes bots, often in large numbers. This treatment was advocated by Perroncito and Bosso in 1894, and subsequently found efficacious by Wessel, Taar, Bugarli, Cognesi, Hanke, Kröning, Nieder and others. It was recommended in the Weekly News Letter of September 10, 1913, issued by the U. S. Department of Agriculture. I have been told that tests of the treatment were conducted by Dr. Cooper Curtice of the Bureau of Animal Industry and its efficacy confirmed. Carbon bisulphide is at present marketed in this country under a proprietary name for use against bots and worms. Against worms it has a certain anthelmintic value, not as yet well ascertained.

The writer's experience has indicated that it is not easy to determine the efficacy of an anthelmintic by the number of worms passed after treatment, since a few worms passed may represent all those present and a large number may represent a small proportion of those present. Consequently it has seemed worth while to apply the acid test for such treatments, *i. e.*, a treatment followed by post-mortem examination, to the carbon bisulphide method, which method may be regarded as anthelmintic in its broad aspects and insecticidal in its intent.

Horse No. 3 was first given anthelmintic treatment consisting of 12 mils of oil of chenopodium, followed at once by 800 mils of linseed oil. The examination of the manure for the next 4 days showed no bots. In these experiments the manure was very carefully examined, being picked apart, a small handful at a time, by myself or my assistant, Mr. B. A. Drake. It was a very long and tedious operation, but such as to warrant us in the belief that no bots present could be overlooked. On the fourth day this horse, which had not been fed since the previous evening, was given 20 mils of carbon bisulphide in a capsule at 9:45 A. M. This was repeated at 11:15 A. M. At 1:45 P. M. the horse was given 800 mils of linseed oil. No bots were found in the manure the next day. The manure for the second and third day was examined the third day and 1 bot was found. The fourth day there were 5 bots, and the fifth day there were four bots. During the next three days no more bots were passed, so on the eighth day the horse was given the carbon bisulphide more nearly in accordance with the method published by the U. S. Department of Agriculture, the animal receiving 3 doses of 3 drams (about 12 mils) each at hour intervals, the treatment being given after fasting the horse from the previous evening. However, the preliminary purgation there recommended was not undertaken. An examination of the manure for the next 16 days did not disclose the presence of a single bot.

On the sixteenth day after the second treatment, the horse was killed. A very thorough examination of the digestive tract, including an examination of all of its contents by methods as careful as those used in examining the manure, showed an entire absence of bots of any sort. The first treatment, resulting in the passage of 10 bots in 5 days, was therefore 100 per cent effective, and would presumably have been that effective if given as recommended by the Department of Agriculture or in four 10- to 12-gram doses at hour intervals as recommended by some writers. It is usually more convenient to give 1 or 2 doses than 3 or 4, all other things being equal, but owing to a certain element of danger in such drugs as carbon bisulphide, small doses given more frequently are safer, since treatment can be stopped if untoward symptoms develop early. An examination of the bots passed showed that all three of the common species, *Gastrophilus intestinalis* (*G. equi*), *G. nasalis* and *G. hemorrhoidalis*, were represented, the treatment being effective against all of them. The stomach of the horse showed some hemorrhagic and necrotic areas and a small ulcer in the cardiac portion. Whether the carbon bisulphide was responsible to any extent for the pathological conditions noted is a question that I would not care to pass on without further data. Various veterinarians have reported bad effects in horses from the administration of carbon bisulphide, and Hutyra and Marek state that some animals may even succumb to it.

Horse No. 4 was given 20 mils of carbon bisulphide, followed in 1½ hours by 800 mils of linseed oil. Five days later this horse was given 12 mils of oil of chenopodium, followed at once by 800 mils of castor oil. Nine days after the chenopodium treatment, the carbon bisulphide treatment was repeated, giving 3 doses of 3 drams each. Ten days after the last treatment the animal was killed. No bots were passed during the entire treatment and none were found post-mortem. This experiment, therefore, throws no light on the question of efficacy. The digestive tract was normal, showing that the treatment does not necessarily involve serious lesions of the digestive tract.

Aside from carbon bisulphide, turpentine is practically the only remedy used to remove bots. Inasmuch as chloroform is a very penetrating and lethal agent, it might be supposed that it would kill bots. In practice it does not appear to have been found of value in this connection, and experimental test supports the idea that it is not useful in expelling bots. Horse No. 2 was given 16 mils of oil of chenopodium, followed immediately by 800 mils of linseed oil and 100 mils of castor oil. No bots were passed subsequent to this treatment, and 15 days later the horse was given 20 mils of chloroform, followed 15 minutes later by 750 mils of linseed oil. No bots were passed during the next six days, and the horse was killed on the sixth day. Post-mortem examination disclosed the presence of 7 bots in the stomach, 35 in the duodenum, and 1 in the cecum. The chloroform was therefore entirely ineffective against bots. The stomach, with the exception of the areas where the bots attached, was normal; the intestines showed local congested and inflamed areas.

We may therefore summarize the data obtained from the above experiments in treatment as follows: Carbon bisulphide is highly effective against all American species of bots (100 per cent in this experiment); chloroform, contrary to what one might suppose, is quite ineffective against bots, in the dose tested and as given; and oil of chenopodium, as one might suppose, is of no value against these highly resistant larvæ. As noted later, chloroform also failed to remove bots from dogs.

Possession of the bots from Horse No. 2 suggested an experiment along the lines of incidental myiasis. Within 5 hours after they were collected, six specimens of *Gastrophilus intestinalis* (*G. equi*) and 6 of *G. nasalis* were put in No. 00 gelatin capsules, 1 to a capsule, and 3 specimens of each species fed to each of 2 dogs, Nos. 93 and 94. Bots, 1, 2, and 3 in number, have been found in the stomach of the dog at least three times, aside from artificial infestations, their occurrence there being attributed to the eating of the bot-infested stomach of the horse. Bots have been reported once from the stomach of the hyena, apparently infested in the same way, and a similar explanation may be invoked in the case of *Gastrophilus inermis* reported as occurring firmly attached in the crop of a crow by Skrjabin. Railliet reported feeding the larvæ of *G. intestinalis* (*G. equi*) to 5 dogs. One had no bots at the end of 8 days; another had 1 bot attached in the stomach and 1 free in the ileum in 20 hours; a third had 5 bots attached in the stomach, 2 attached in the rectum and 2 free in the rectum at the end of 7 days; a fourth had 2 larvæ attached to the stomach at the end of 15 days; while a fifth had no bots a month after being fed 6 of them.

In our experiment the feces from the 2 dogs were carefully examined each day, being comminuted and washed through the set of screens which the writer has described in previous publications. Dog No. 93 passed 4 bots on the third day after he ingested them; on the twenty-fourth day he passed another bot and some *Dipylidium.* On the twenty-fourth day the dog was given 10 minims of oil of chenopodium, 20 minims of chloroform and 30 mils of castor oil, followed 2 hours later by 60 mils of castor oil. The following day the dog passed 1 whipworm and the next day 3 whipworms. On the day preceding the anthelmintic treatment the dog had passed 1 ascarid. On post-mortem examination, 35 days after the ingestion of the bots and 11 days after the anthelmintic treatment, 1 live bot, *Gastrophilus intestinalis,* was found in the tip of the cecum, apparently not attached. There were also 7 whipworms in the cecum and 2 hookworms in the small intestine.

Dog No. 94 passed 2 bots on the eighth day after ingesting them, and passed another on the ninth day, the last one being accompanied by the passage of 3 whipworms. On the twenty-fourth day the dog was given 15 minims of oil of chenopodium, 30 minims of chloroform and 60 mils of castor oil, followed 2 hours later by 30 mils of castor oil. On post-mortem examination, 35 days after the ingestion of the bots and 11 days after the anthelmintic treatment, 1 live bot, *G. intestinalis*, was found attached in the fundus of the stomach, and another live specimen of the same species was found attached to the large intestine about 6 inches from the anus. There were 125 whipworms present in the cecum. The sixth bot was unaccounted for; it had probably been passed alive and had crawled out of the pan forming the floor of the dog's cage.

From the foregoing it appears that of 6 specimens of *Gastrophilus intestinalis* fed to 2 dogs, 3 specimens succeeded in establishing themselves for the 35-day period, which terminated in the death of the dogs. They were established in such diverse locations as the stomach, the cecum and the lower part of the large intestine. The one in the stomach was undoubtedly well established; the others may represent larvæ which had formerly attached in the stomach but were unable to maintain that position and were holding at different points on their way out. There is no obvious reason why bots of species other than *G. intestinalis* should not be found in the stomach of the dog; at the same time this experiment suggests that there may be some reason for this. None of the specimens of *G. nasalis* which were fed to these dogs maintained themselves up to the time of post-mortem examination. They were evidently able to maintain themselves as much as 8 days, and might have stayed as much as 24 days (the species of the bots as passed was unfortunately not ascertained), but they failed to establish themselves as did *G. intestinalis*. The reason for this is not at all evident. They were apparently no more mature, which would have been a reason for passing out. *G. intestinalis* usually lives in the esophageal portion of the stomach of the horse, whereas *G. nasalis* and *hemorrhoidalis* usually live in the pyloric region or in the duodenum, but I am unable to formulate a reason from this as to why *G. intestinalis* should survive in the stomach and intestine of the dog and the others fail to do so.

The above experiments were performed in March. In September, Dog No. 129 was given 6 full-grown specimens of *G. intestinalis* and 6 young specimens of *G. hemorrhoidalis,* in capsules. He presently vomited the 6 *G. intestinalis* and 3 *G. hemorrhoidalis.* These were given again, and presently the dog vomited 3 *G. intestinalis* and 2 *G. hemorrhoidalis,* which were again given to him. The day following, the dog had passed 2 *G. intestinalis;* the third day, he passed a third bot of this species; the fourth day, a fourth; and the thirteenth day, a fifth bot of this species. The twenty-ninth day after giving the bots to the dog the animal was killed, and no bots were found on post-mortem. It is likely that the bots not recovered were vomited during the first 24 hours, and being still lively had escaped from the feces and the cage. At any rate, young specimens of *G. hemorrhoidalis* failed to establish themselves in the dog. They certainly failed to remain a month and apparently failed to establish themselves at all. The short time the *G. intestinalis* remained, 13 days or less, may be correlated with the season, the bots being fully-developed, and perhaps incapable of overwintering in a host.

It is interesting to note that the passage of one bot from Dog No. 93 was preceded the day before by the passage of 1 ascarid, was accompanied by the passage of *Dipylidium,* and was followed the next day by the passage of 1 whipworm and the second day by the passage of 3 whipworms; and that the passage of 2 bots from Dog 94 was followed the next day by the passage of 3 whipworms, accompanied by another bot. In the case of Dog. No. 93, the dog was given anthelmintic treatment the day the bot passed, and this might account for the subsequent passage of whipworms, but the passage of the ascarid and *Dipylidium* in this case and of the whipworms in the case of the other dog suggest that the bots acted as mechanical anthelmintics, entangling *Dipylidium* and whipworms in their spiny covering and perhaps driving the ascarid along as a result of contact with these spines. It is possible that some cause operated to expel the worms and bots together, but such spontaneous expulsion of such dissimilar parasites from 2 dogs is a less likely explanation than that the bots acted as mechanical anthelmintics. The frying of tow or oakum in grease and feeding it to a dog as a mechanical anthelmintic is an old device; even cowhage and similar substances have been recommended as mechanical anthelmintics, though probably less effective than fried oakum. One might even include among mechanical anthelmintics a tapeworm trap, recorded in the U. S. Patent Office, which is intended to be baited for tapeworm and swallowed by the patient.

In a recent article, Roubaud (1917: Compt. rend. Acad. d. Sci., v. 164 (11), 12 Mar., pp. 453-456) has made some very interesting observations in regard to the life history of the bot-fly, *Gastrophilus intestinalis* (*G. equi*). He notes that some authors think that the larvæ escape from the egg and gain the mouth and nose; most writers think that they get into the mouth as a result of the horse licking the hairs and are then swallowed, some writers explaining the licking as following a slight pruritus due to the action of these larvæ on the skin; reasoning from the presence of *Gastrophilus* larvæ in the skin of man, Cholodkovsky believes that the larvæ leave the egg and penetrate the skin of the horse, causing an itching which leads the horse to scrape these parasitic burrows with his teeth, thereby rooting out and ingesting the larvæ; reasoning from the same starting point, Portchinski believes that only some of the larvæ, individuals sacrificed for the preservation of the species, penetrate the skin of the horse, the irritation resulting in the horse licking off other larvæ which are not embedded in the skin and swallowing them.

Roubaud has evidence pointing to a different life history. According to his observations, the eggs of the bot-fly do not open spontaneously and the primary larvæ may remain in the egg for several weeks; Guyot has observed cases where they lived thus over 3 months. Roubaud finds that a brisk shock on the anterior pole of the egg will cause the operculum to open, the larvæ promptly escaping from the shell; moisture, such as would be furnished by licking, is not necessary. This conforms, moreover, to the well-known fact that horses lick themselves with the tongue very little; when a horse has an itching or irritated area, he rubs it with the nose or bites it. The larvæ probably escape when a horse does this for any cause whatever. The most interesting feature of Roubaud's observation, however, is the fact, demonstrated by bot larvæ on guinea-pigs, that when the larvæ escapes from the egg and is brought in contact with the buccal mucosa, it promptly burrows into the mucosa and lies parallel to its surface in a relatively superficial position. When brought in contact with the skin of the horse, whether it is wet or dry, these larvæ do not burrow and do die very rapidly on the dry skin. He thinks bot larvæ in the human skin enter through the lips or eyelids or through a lesion in the skin, and notes that eggs of *G. hemorrhoidalis* have been found on the eyebrows and lashes in such cases of cutaneous myiasis. Larvæ under the buccal mucosa disappear in 2 or 3 days, but one was seen traveling along the side of the tongue for 9 days, during which time it grew to three times its first dimensions. The larvæ probably undergo the molt to the second stage at this time and leave the buccal epithelium for the gastric cavity.

During recent years we have seen our ideas regarding the life history of the ox warble, *Hypoderma,* upset; Roubaud's findings apparently necessitate a certain revision of our life history for the bot flies.

SUMMARY. Carbon bisulphide, in experimental test followed by post-mortem examination, is as highly effective against bots as clinical findings indicate.

Neither chloroform nor oil of chenopodium are of any value in removing bots, so far as our experiments show; this bears out the clinical findings for chloroform and conforms to the probabilities for oil of chenopodium.

Gastrophilus intestinalis (*G. equi*) is capable of incidental myiasis in dogs, when well developed larvæ from the stomach of the horse are fed to them, but *G. nasalis* and *G. hemorrhoidalis* seem to have only a limited capacity for adaptation to conditions in the dog.

Roubaud's investigations indicate that the bot larva escapes from the egg when the horse bites or rubs his skin with his mouth and burrows into the buccal mucosa before proceeding to the stomach.

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Studies from the Research Laboratory
Parke, Davis & Co.
Reprint No. 138, 1917.

(Reprint from *The Journal of Laboratory and Clinical Medicine*, Vol. III, No. 2, November, 1917.)

AN INVESTIGATION OF THE CHEMICAL COMPOSITION AND BIOLOGIC AVAILABILITY OF PEPTONE.*

By Lewis Davis, S.M., Detroit, Mich.

The use of peptone in the preparation of bacteriologic culture media is advocated in the earliest works on bacteriology. While it is undoubtedly a fact that for many organisms ordinary beef infusion furnishes all of the necessary food requirements, addition of even a small amount of peptone causes a pronounced increase in growth and metabolic activity.

Bainbridge[1] and others have observed that even some of the extremely active putrefactive organisms are unable to attack and decompose native proteins. However, if a small quantity of peptone or some other readily assimilable, nitrogenous material be present, decomposition soon takes place with rapid disappearance of the protein.

That there is a specificity to the role of peptone in a culture medium is particularly shown by its use in the elaboration of toxin by Bact. diphtheriæ, the production of indol by the colon bacillus and its necessary presence for the successful growth *in vitro* of such delicate organisms as the spirochetes. The early investigations of Park and Williams[2] have pointed out the necessity of peptone in the production of diphtheria toxin. Recent successful use of media containing tryptophane[3] for the production of indol by bacteria clearly shows that the function of the peptone in Dunham's medium is to furnish material containing tryptophane.

It is readily apparent that the chemical composition of peptone has a decided influence on its bacteriologic availability. That

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this question has not received more attention in the past is largely due to the fact, that almost from the beginning of bacteriology, Witte's peptone has served as standard in culture media preparation. The present scarcity of this imported peptone and the appearance of a number of domestic substitutes have necessitated careful consideration of their biologic availabilities. The present investigation was undertaken to compare the chemical composition as well as the utilization by bacteria of domestic peptones with Witte's peptone as a standard. In the course of the study, data was obtained which finally led to the preparation of an experimental peptone that has been found entirely satisfactory for bacteriologic use and in the production of toxins.

EXPERIMENTATION

A. *Method of Analysis*

Samples of the various brands purchasable in the open market were obtained, each given a laboratory number for identification, and the examination was then conducted under the following captions:

1. *Physical examination,* including appearance, odor, solubility in cold and hot water and preparation of the more common culture media from each sample, in accordance with the Standard Methods of the A. P. H. A.[4]

2. *Chemical examination* comprised analyses of total nitrogen, moisture, total mineral matter, phosphoric acid as P_2O_5, chlorides as NaCl, calcium as CaO and determination of reaction in aqueous solution. Supplementing the above, qualitative determinations were made for the presence of albumoses (addition of saturated zinc sulphate, ammonium sulphate, picric acid solutions) protoproteoses (addition of saturated sodium chloride solution, potassium ferrocyanide in acetic acid solution), tyrosin (xanthoproteic, Millon's reaction); tryptophane [(Adamkiewicz)-Hopkins-Cole reagent]; creatinin (Jaffe's reaction), the behavior towards the biuret test, as well as with three volumes of 95 per cent alcohol and a comparison of free amino acids present.

3. *Bacteriologic examination,* made in association with my colleague, H. C. Ward, included the determination of the conduct of various bacteria in the more common liquid and solid

media made from each sample, growth and production of indol in Dunham's solution, viability tests with sensitive organisms, and direct isolation of bacteria from pathologic material. Of greater delicacy than any of the preceding tests, the writer has found to be the production of a potent toxin by B. diphtheriæ in ordinary (2 per cent) peptone bouillon. In fact, this test combined with development of indol by B. coli in Dunham's solution has been employed as "a preliminary test" of bacteriologic availability in the examination of experimental peptones.

For the analysis of total nitrogen, a modified Gunning method was used, which, while giving somewhat lower results than the official methods, was simpler and permitted of comparative results with equal accuracy. Moisture and total mineral matter were determined in platinum utensils in accordance with the usual technic. Phosphoric acid was estimated in a nitric acid solution of the ash by precipitation as ammonium phosphomolybdate, and dissolving in an excess of sodium hydroxide as employed in the volumetric estimation of total phosphoric acid in fertilizers.[5] The same solution of the ash was used for volumetric determinations of chlorides and calcium. The calcium was precipitated as oxalate, dissolved in sulphuric acid and titrated against potassium permanganate solution, while the solution for chlorides was carefully neutralized with sodium hydroxide, and then titrated against silver nitrate solution with potassium chromate as indicator by Mohr's method.[6]

Estimating proteoses by precipitation with saturated zinc sulphate solution according to Bömer[7] was tried and gave fairly concordant results with Witte's peptone which, as is known, consists largely of proteoses. Most of the other samples examined had so little albumoses present that qualitative examination with the reagents mentioned above gave all the necessary information. Direct estimation of peptone nitrogen by phosphotungstic acid or bromine precipitation was found to be unsatisfactory. As the "tannin-salt method of Sjerning, as modified by Bigelow and Cook,[8] is dependent upon the proteose nitrogen value, this procedure could not be accurately applied for peptone valuation. Here again the qualitative examination, supplemented by the biuret test, gave some idea of the degree of digestion of the sample.

The reaction of the sample peptones was determined on a 1 per cent aqueous solution both by a hot titration method and more accurately with the hydrogen electrode. For the hot titration, 10 c.c. of the 1 per cent peptone solution were diluted with 40 c.c. of water, boiled for one minute, then titrated hot against N/10 sodium hydroxide, using phenolphthalein as an indicator. The concentration of hydrogen-ions was measured by the hydrogen electrode recommended by Bovie,[9] using a Weston Standard Cell, calomel electrode, galvanometer, and the direct reading potentiometer described by Bartell.* Comparison of the free alpha amino acids present in Witte's and the other test peptones was made with 1 per cent solutions, using the colorimetric method of Harding and MacLean[10] (color produced by twenty minute boiling with pyridine and ninhydrin) and a Duboscq colorimeter.

As test organisms for determining the nutrient index of peptone samples by subculture transfers, B. coli, staphylococcus aureus, diplococcus meningitis, streptococcus pyogenes, M. catarrhalis, M. gonorrhœæ and B. prodigiosus were employed. The growths on slant agar at 37° C. both after 24 hours and 48 hours were recorded for all except B. prodigiosus, which was cultivated at 20° C. Inoculations from these were made to a new series of agar slants and the growths noted, after which the above process was twice repeated.

Dunham's solutions containing the test peptones (with 0.5 per cent sodium chloride) and sterilized at autoclave temperature, were used for growth and viability tests. Vigorous 24-hour, slant agar cultures of Staph. aureus, B. coli, and B. prodigiosus were emulsified with 10 c.c. of salt solution, filtered, and a uniform amount of suspension inoculated into a series of test peptone tubes by mixing with capillary and bubbling. A 1/10 c.c. sample was then removed from each tube and agar plates made as "controls." These were incubated at the optimum temperatures and counted. The peptone tube cultures were now grown for 24 hours and plates again made as before, the same procedure being repeated at 72 hours and 96 hours.

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The work on hydrogen-ion concentration was done at the University of Michigan, Ann Arbor, in the laboratory of Prof. Bartell, to whom the writer's sincere thanks are due.

For the indol valuations, the same (Dunham's peptone) solutions as above were employed. Four cultures of B. coli of known indol-producing capacity (with Witte's peptone) were selected, together with a culture of B. typhosus as a "negative control." Uniform, comparable, suspensions in sterile salt solution of a 24-hour slant agar culture of each of the organisms were made and 1 c.c. of each culture suspension added to sets of sample peptone tubes. The tubes were incubated at 37° C. for 96 hours, then qualitative indol determinations were made by adding 1 c.c. of a 10 per cent sulphuric acid solution, thoroughly mixing, and then adding without mixing 1 c.c. of a freshly made 0.01 per cent solution of sodium nitrite. The formation of the well-known purplish-red, nitroso-indol ring of Salkowski[11] on standing in the cold for fifteen minutes indicated presence of indol, a record being made of the intensity of the ring. Although this test is not as delicate as the modified test of Ehrlich,[12] is was deemed of more practical significance, since for the case in hand indol should be formed in appreciable quantity to be of value as a diagnostic index.

Comment has already been made upon the importance of diphtheria toxin production in valuing a peptone sample. The test culture employed was of known toxin-producing power and gave rapid and abundant pellicle formation in ordinary 2 per cent peptone (Witte) bouillon. A reaction of the bouillon corresponding to a hydrogen-ion concentration of 1×10^{-8}* (+5 on the Fuller Scale) before sterilization was found to give the best results. On sterilizing such a medium in an autoclave at 115° C. for 20 minutes, the reaction rises to about +10 on the Fuller Scale ($C_{H^+} = 3 \times 10^{-8}$), which permits of rapid and vigorous growth.

In order to accustom the organism to any variations due to the test peptone, "starter" flasks containing 30 c.c. of bouillon in a 250 c.c. flask were first inoculated, cultured for 24 hours at 37° C., the purity of the growths checked, and the contents aseptically transferred to 3 liters of the same bouillon in 6 liter flasks. After incubating for 12 days at 37° C., examination for purity was made, 0.4 per cent trikresol added, allowed to stand 24 hours, then filtered.

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*The relationship of hydrogen ion concentration to toxigenicity of Bact. diphtheriæ is being studied, and will appear in a later paper.

Since diphtheria toxin is usually employed for immunization purposes, the strength of the toxins prepared as above was estimated by the L+ dose in accordance with the Hygienic Laboratory Method.[13] An interesting fact noted in cultivating Bact. diphtheriæ for toxin production was, that while the growth in larger flasks showed no gross difference from that obtained in small flasks, other conditions being the same, there was a marked variation in both toxicity and final reaction. Thus, 500 c.c. of bouillon in a liter flask invariably gave a weaker toxin and final alkaline reaction to phenolphthalein, while 3000 c.c. in a six liter flask gave a stronger toxin with final acid reaction to phenolphthalein. Large flasks were accordingly employed for all toxin cultivation with test peptones.

No preliminary cultivation was found necessary for the production of tetanus toxin. A virulent culture of B. tetani was used in glucose bouillon (2 per cent peptone) with oil, incubation carried on for three weeks at 37° C., examination for purity then made, and the same procedure followed as with diphtheria toxin. In accordance with the usual practice, the strength was determined by an approximate estimation of the minimum lethal dose, as recommended by Rosenau and Anderson.[14]

B. *Laboratory Results.*

Exclusive of experimental products, seven different brands of bacteriologic peptone were examined. These comprised samples of Witte's and six domestic products which are grouped in the succeeding tables of results under the caption of "American Brands." In addition to the preceding, there are included in the comparisons two experimental products designated as "Exp. No. 1" and "Exp. No. 2," respectively. The first of these two peptones, after the basic constituents had been determined upon, was devised so as to give a product having as nearly as possible the same general reactions and gross chemical composition as Witte's peptone. Further work on bacterial utilization of peptones showing, among other factors, greater degrees of hydrolysis than experimental No. 1, led to the preparation of the second of the two experimental products given in the tables. In all cases

TABLE I.

PROPERTIES OF PEPTONE SAMPLES.

AMERICAN BRANDS.

	No. 51	No. 67	No. 125	No. 126	No. 132	No. 138	Witte	Exp. No. 1	Exp. No. 2
Appearance	Granular, cream yellow powder	Fine, cream yellow powder	Fine, cream yellow powder	Coarse, yellow powder	Fine, cream colored powder	Fine, brown powder	Fine, cream yellow powder	Fine, cream powder	Fine, cream powder
Odor	Slight putrid.	Mild, not unpleasant	Rancid, unpleasant	Pleasant, sweetish	Mild, unpleasant	Burnt meat	Stale, unpleasant	Mild, not unpleasant	Mild, not unpleasant
Solubility (2%) in water	Readily in cold, colloidal turbidity. Same on boiling.	Readily in cold, turbid solution. Ppt. on boiling	Readily in cold, slight turbidity. Ppt. on boiling	Readily in cold, slight turbidity. Ppt. on boiling	Readily in cold, turbid solution. Ppt. on boiling	Readily in cold, turbid solution. Same on boiling	Difficulty in cold, more easily on boiling. Heavy residue	Easily in cold, turbid solution. Moderate ppt. on boiling	Easily in cold, clear solution. Slight ppt. on boiling
Behavior in media preparation	Filters slowly	Filters readily. No difficulties	Filters readily. No difficulties	Filters readily	Filters readily. No difficulties	Filters with difficulty	Filters readily. No difficulties	Filters readily. No difficulties	Filters readily. No difficulties
Bouillon (appearance)	Dark colored, turbid	Dark colored, clear	Light colored, clear	Dark colored, clear	Light colored, clear	Dark colored, turbid	Light colored, clear	Light colored, clear	Light colored, clear
Agar (appearance)	Dark colored, clear	Dark colored, clear	Light colored, clear	Dark colored, clear	Light colored, clear	Dark colored, clear	Light colored, clear	Light colored, clear	Light colored, clear
Dunham's solution (appearance)	Slight turbidity, light yellow	Clear, light yellow	Clear, light yellow	Clear, yellow	Clear, yellow	Clear, light yellow	Clear, almost colorless	Clear, almost colorless	Clear, almost colorless

TABLE II.

REACTIONS OF PEPTONE SAMPLES IN 1 PER CENT SOLUTION.

AMERICAN BRANDS.

	No. 51	No. 67	No. 125	No. 126	No. 132	No. 138	Witte	Exp. No. 1	Exp. No. 2
Xanthoproteic	No ppt. with HNO_3. Light yellow color. Orange+NaOH	No ppt. with HNO_3. Light yellow color. Orange+NaOH	No ppt. with HNO_3. Light yellow color. Orange+NaOH	No ppt. with HNO_3. Light yellow color. Orange+NaOH	No ppt. with HNO_3. Light yellow color. Orange+NaOH	No ppt. with HNO_3. Light brown color. Orange+NaOH	No ppt. with HNO_3. Deep yellow color. Orange+NaOH	No ppt. with HNO_3. Yellow color. Orange +NaOH	No ppt. with HNO_3. Yellow color. Orange +NaOH
Sat. Picric Acid Sol.	Moderate amorphous ppt.	Moderate amorphous ppt.	Slight ppt.	Slight ppt.	Slight ppt.	Slight ppt.	Heavy amorphous ppt.	Heavy amorphous ppt.	Moderate amorphous ppt.
Sat. Ammon. Sulph. Sol.	Slight ppt.	Slight ppt.	Trace of ppt.	Slight ppt.	Trace of ppt.	Slight ppt.	Heavy ppt.	Moderate ppt.	Slight ppt.
Sat. Zinc Sulph. Sol.	Slight ppt.	Small ppt.	Trace of ppt.	Slight ppt.	Trace of ppt.	Trace of ppt.	Heavy ppt.	Moderate ppt.	Slight ppt.
Millon's	Small ppt. Red on boiling	Heavy ppt. Deep red on boiling	Small ppt. Red on boiling	Moderate ppt. Red on boiling	Small ppt. Red on boiling	Heavy ppt. Deep red on boiling	Moderate ppt. Red on boiling	Heavy ppt. Deep red on boiling	Slight ppt.
Biuret	No ppt. with $CuSO_4$. Red color + KOH	No ppt. with $CuSO_4$. Deep pink + KOH	No ppt. with $CuSO_4$. Deep pink with KOH	No ppt. with $CuSO_4$. Old rose with KOH	No ppt. with $CuSO_4$. Purple pink + KOH	No ppt. with $CuSO_4$. Pink + KOH	Small ppt. with $CuSO_4$. Pink color + KOH	No ppt. with $CuSO_4$. Pink + KOH	No ppt. with $CuSO_4$. Deep pink + KOH
Glyoxilic. Hopkins-Cole.	Strong ring	Small ring	Small ring	Strong ring	Very weak ring	Very weak ring	Strong ring	Very strong ring	Very strong ring
Sat. sod. Chloride Sol.	No ppt.	No ppt.	No ppt.	No ppt.	No ppt.	No ppt.	Slight ppt.	No ppt.	No ppt.
Pot. Ferrocyanide + Acetic Acid	Slight ppt.	No ppt.	No ppt.	No ppt.	No ppt.	No ppt.	Slight ppt.	No ppt.	No ppt.
95% Alcohol (3 vols.)	Slight ppt.	Trace of ppt.	Trace of ppt.	Trace of ppt.	Trace of ppt.	Trace of ppt.	Small ppt.	Small ppt.	Small ppt.
Sat. Picric Acid + NaOH	Yellow color	Orange color	Yellow color	Red orange color	Slight orange color	Strong red orange color	Yellow color	Yellow color	Yellow color

the results tabulated in the foregoing are based on at least three separate samples of each brand, duplicate determinations having been made with each sample.

The physical examination, as may be noted from Table I, shows the general appearance of the various brands to be nearly the same. Only two products (No. 51 and No. 125) evidenced a decidedly unpleasant odor, the odor in one case (No. 51) being apparently due to microbial decomposition, as the moisture content of this sample was high.

A marked difference of solubility in water (2 per cent solution) is found between Witte's peptone and all the other brands examined. While the former dissolves only with difficulty in cold water and leaves a bulky residue on boiling, all of the other products readily go into solution in the cold, and leave only a small precipitate on boiling. Results obtained with experimental samples indicate that this residue given by Witte's peptone is partly due to its higher content of proteoses and other protein degradation products. In two cases (No. 51 and No. 138) the precipitate obtained was colloidal in nature, filtered with difficulty, and gave turbid bouillon and Dunham's solution, but had no effect on the agar. Media prepared from four of the samples (Nos. 51, 67, 126, and 138) were considerably more darkened than would be expected as a result of autoclave sterilization.

Table II shows that all of the brands examined give a strong xanthoproteic reaction indicating presence of tyrosin, which is confirmed by the behavior with Millon's reagent. Tryptophane is shown by a strong Hopkins-Cole (Adamkiewicz) reaction in Witte's peptone, both experimental products, No. 51 and No. 126. Creatinine, as given by Jaffe's reaction, is present in perceptible amount in No. 138, and to a less extent in Nos. 67, 126, and 132. Excluding the first experimental product which was prepared so as to contain albumoses in quantity, Witte's peptone is the only product showing an appreciable portion of albumoses as measured by the reactions with saturated ammonium sulphate, zinc sulphate and picric acid solutions. A small amount of primary proteoses in Witte's peptone is revealed by the precipitates obtained with copper sulphate (biuret test) and on addition of potassium ferrocyanide solution to an acetic acidified solution of the peptone. That this substance is not present in appreciable quantity is shown by the absence of precipitate with the saturated sodium chloride reagent.

Both the biuret reaction and the behavior towards three volumes of 95 per cent alcohol were used as comparative tests to determine similarity with Witte's peptone rather than to detect any specific substance. Although the "biuret" color obtained with most of the samples, when the reaction was performed under uniform conditions, might be characterized as pink, there was still a considerable difference in the intensity of this color. From experimental preparations, it appears possible that the color factor is influenced by the degree and manner of hydrolizing the constituent proteins. All of the American brands appear to be nearly completely soluble in three volumes of 95 per cent alcohol, while both Witte's and the experimental products show small precipitates with this reagent.

TABLE III.

Analyses of Peptone Samples.

	AMERICAN BRANDS.								
	No. 51	No. 67	No. 125	No. 126	No. 132	No. 138	Witte	Exp. No. 1	Exp. No. 2
Total Nitrogen	10.91%	13.50%	13.78%	12.41%	15.56%	12.42%	14.52%	14.35%	14.25%
Moisture	8.84%	5.52%	5.08%	10.20%	4.82%	6.43%	6.35%	5.13%	4.3 %
Ash	13.71%	8.20%	3.98%	13.01%	3.92%	10.13%	3.44%	4.06%	2.86%
Phosphoric Acid as P_2O_5 in ash	0.18%	0.96%	1.66%	1.64%	1.05%	2.11%	0.42%	0.75%	1.02%
Calcium as CaO in ash	0.80%	0.45%	0.82%	0.52%	0.51%	0.70%	1.08%	1.13%	0.10%
Chlorides as NaCl in ash	7.76%	3.72%	0.13%	5.67%	0.26%	2.39%	0.54%	0.72%	0.35%
Reaction (1% sol.) Fuller Scale	+9	+13	+15	+14	+11	+23	+6	+10	+13
H-ion Con'n (1% sol.)	7.6x 10^{-7}	5.0x 10^{-6}	6.1x 10^{-7}	5.8x 10^{-7}	4.3x 10^{-6}	8.0x 10^{-6}	3.2x 10^{-7}		6.8x 10^{-7}
Ratio Amino Acids (Witte=1)	1.07	1.75	2.05	2.00	1.75	3.12	1.00		2.22

The analytical data presented in Table III shows that not only is there a marked variation among the domestic products themselves, but also a difference from Witte's and the experimental products. The total nitrogen ranges from 10.91 per cent (No. 51) to 15.56 per cent (No. 132) with the "normal" as given by Witte's peptone, about 14.5 per cent. Moisture seems to be a more nearly constant factor, the average being about 6 per cent. and only two samples (No. 51 and No. 126) show amounts above 8 per cent. These same samples also have the highest content of mineral matter, both over 13 per cent, and the lowest amount is shown by experimental No. 2 with less than 3 per cent. In all cases the high ash content is found to consist mostly of chlorides, probably sodium chloride, and with one exception (No. 51), phosphates in addition. Calcium appears to be a predominant constituent in the ash of Witte's peptone and also in the first of the experimental products. Most of the domestic samples appear to have this cation present in only about half the amount shown by the two brands mentioned.

Comparison of the acidity which 1 per cent aqueous solutions of the various brands give by the hot titration method shows Witte's peptone to have a value of +6 (Fuller Scale) against more than twice this amount recorded for nearly all of the other brands. The fallacy of "hot titration" and the errors in the use of phenolphthalein as an indicator for culture media reactions have been ably pointed out by Clark.[15] Determination of the actual hydrogen-ion concentration of these peptone solutions by the gas cell show three brands (Nos. 67, 132, and 138) to have what might be termed an appreciable acidity, above 4 x 10^{-6}, while the remainder are very nearly neutral. It is interesting to note, as substantiating the findings of Clark, that two of these three more acid peptones, Nos. 67 and 132, actually show *less* acidity by hot titration (+13, +11) than do peptones of smaller hydrogen-ion concentration, like No. 125, and No. 126, which values of +15 and +14, respectively (Fuller Scale).

The fact that Witte's peptone, as may be noted from Table II, is the least digested of any of the other brands examined, suggested the possibility that the acidity of a peptone solution when valued by hot titration is intimately associated with the amount of free amino acids present. Using Witte's peptone as a standard, colorimetric comparison of the amount of free amino acids in the various samples shows this to be the case. In other words, the high titration values are given by the products having higher amounts of free amino acids present. Aside from peptone No. 138 and Witte's, which represent the two extremes, the true reaction of the other products, as measured by their hydrogen-ion concentration, appears to bear no relationship to the free amino acids present.

In determining the nutritive value of peptones for general bacterial requirements, biologic tests are desirable which will give a definite, quantitative estimation. As a consequence, subculture transfers on slant agar in accordance with the method already outlined gave no satisfactory comparisons. The more delicate organisms, like the meningococcus, M. catarrhalis and M. gonorrhœæ, showed no viability on any peptone agar after the fifth or sixth transfer, while the more common semiparasitic types, which are not as complex in their food requirements, gave luxuriant growth with all of the peptones examined.

Attempts to value the peptone samples by agar plate estimations, as described under "Methods of Analysis," were abandoned. The organisms employed grew so luxuriantly in Dunham's solutions, made from all of the test peptones, that flocculent precipitates were formed which interfered with accurate counts by the place method. Direct isolation of bacteria from pathologic material by use of media incorporating the test peptones appears to be a more promising index of nutritive value. This, however, is necessarily a slow process and comparative data to be used for this purpose is still accumulating. In view of the preceding, more significance was attached in valuing a sample to its utilization in the production of toxins and also, but to a less extent, in the formation of indol. It is appreciated that bacteriologists do not regard indol formation, *per se,* as of paramount importance, yet it will be conceded that a peptone which does not permit of indol production would not be considered as practical for bacteriologic application. Aside from the practical importance of toxins in the production of antitoxins and of indol formation in the sense just discussed, both of these products are capable of quantitative measurement.

The results given in Table IV show that only one of the domestic brands (No. 126) when incorporated into bouillon permits of vigorous growth and pellicle formation by the diphtheria bacillus. Luxuriant growth and heavy pellicle formation by Bact. diphtheriæ do not necessarily indicate strong toxin production as shown by the fact that the toxin in this case required

TABLE IV.

BACTERIAL UTILIZATION OF SAMPLE PEPTONES.

Peptone Test	Growth B. Diphtheriæ in 2% Bouillon	L + Dose Diphtheria Toxin	Growth B. Tetani Glucose Bouillon	M. F. Dose Tetanus Toxin	Growth B. Coli in Dunham's Sol.	Indol Production B. Coli in Dunham's Sol.
No. 51	Slow—Thin pellicle	Above 1.5 c.c.	Vigorous	0.33 c.c.	Heavy	Moderate
No. 67	Scant—No pellicle	Above 1.5 c.c.	Vigorous	0.25 c.c.	Heavy	Faint
No. 125	Moderate—Thin pellicle	Above 1.5 c.c.	Vigorous	0.25 c.c.	Heavy	Small
No. 126	Rapid and vigorous. Heavy pellicle	1.5 c.c.	Vigorous	0.25 c.c.	Heavy	Strong
No. 132	Scant—No pellicle	Above 1.5 c.c.	Vigorous	0.25 c.c.	Heavy	Faint
No. 138	Scant—No pellicle	Above 1.5 c.c.	Vigorous	0.25 c.c.	Heavy	Faint
Witte	Rapid and vigorous. Heavy pellicle	0.33 c.c.	Vigorous	0.25 c.c.	Moderate	Moderate
Exp. No. 1	Rapid and vigorous. Heavy pellicle	0.40 c.c.	Vigorous	0.25 c.c.	Heavy	Very strong
Exp. No. 2	Rapid and vigorous. Heavy pellicle	0.15 c.c.	Vigorous	0.25 c.c.	Heavy	Very strong

1.5 c.c. for an L + dose. Witte's peptone, as is known, also gives good growth, but a toxin of almost five times the strength (L + dose 0.33 c.c.). The most potent product, however, was furnished by "Experimental No. 2" with an average L + dose less than half that of Witte's (0.15 c.c.). Individual tests of toxins prepared with peptones made in a similar way have shown even higher toxicities (L + dose 0.10).

All of the products examined permit of vigorous growth and strong toxin formation with B. tetani. Tests with experimental preparations show that many hydrolized proteins have this capacity. As may be further noted from the table, heavy growth of B. coli was obtained with all of the products except Witte's when used in Dunham's media. With the Witte samples, the growth is moderate and diffused throughout the tube, and has no tendency toward precipitation as found with some of the other products. Corroborating the tests made for tryptophane given in Table II, the experimental products are found to give the strongest indol tests, with No. 126 next, while Witte's and No. 51 follow, both showing about the same intensity of color formation. Here again, as with diphtheria toxin, luxuriance of growth is found to be no criterion of indol formation.

DISCUSSION.

Consideration of the data presented in the preceding brings into question the value of the examinations, and the significance of the results obtained. In the final analysis, the value of any product must be gauged by the manner in which it fulfils *practical* requirements. With a substance like peptone, the point at issue is not whether it is in granular form or powdered, not whether it has a certain quantity of total nitrogen or chlorides, but how does it on practical test meet the needs of the bacteriologist. In other words, the product, to be of value, must contribute to the nutritive requirements of the ordinary saprophytic and parasitic organisms and yet be of sufficient flexibility in its constitution so as to furnish potent toxins or permit of tracing decomposition changes produced by an organism, as for example the elaboration of indol or the production of hydrogen sulphide. Illustrative of these versatility requirements is the fact that Witte's peptone is employed to advantage in the preparation of the complex media required for cultivating the spirochetes.

Based on these premises, the actual bacteriologic application of test peptones by incorporation in culture media is the crucial test of value. Aside from giving information as to the appearance of the media, the physical properties of a peptone are only of passing importance. Quantitative chemical analyses for the *gross* constituents are merely of value as means of controlling uniformity, once the general requirements for satisfactory peptone are established. Thus, a big deficiency in nitrogenous constituents as measured by total nitrogen, an actual, excessive acidity, or a large amount of mineral matter are undesirable factors revealed by such analyses. It must be borne in mind, in this connection, that the usual concentration in which the peptone is to be employed rarely exceeds 2 per cent.

More important information as to the applicability of a peptone for bacteriologic purposes is furnished by the qualitative examination for amino acids and other hydrolysis products. All protein bodies may be regarded as built up of a certain number of "bricks," of which amino acids play the most important part. Peptone, which is a protein degradation product actually has present a certain amount of amino acids in the free state (Compare Table III), the amount depending on the method of preparation employed.

The value of the peptone in bacterial nutrition will be governed entirely by the "bricks" present which can be utilized by the bacteria. While some of these basic constituents are absolutely necessary for the maintenance of bacterial life and development, others in turn will be utilized, if present, for the production of certain, so to speak, "by-products," not essential to metabolism. Thus, as shown by the results given in Tables II and IV, the products containing appreciable quantities of tryptophane are found to give marked indol formation with B. coli. This, in a measure, also explains why Bact. diphtheriæ can grow luxuriantly as in No. 126 without strong toxin formation. The results obtained in the study at hand show that, among others, tyrosin, as well as tryptophane, is an important constituent of a satisfactory bacteriologic peptone.

The bacteriologic data presented in Table IV show that the domestic products examined give either weak or no diphtheria toxin, while Witte's gives a decidedly potent product. On the basis of the above statements, this should be due to the presence of "bricks" in Witte's which are not present in the other brands. The validity of this hypothesis is shown by the fact that an experimental product (Experimental No. 1) devised so as to contain as nearly as possible the basic constituents of Witte's peptone and hydrolized to the same degree, gives very nearly the same results on practical, bacteriologic, and toxin tests.

From theoretical considerations the more simple the essential basic constituents are, the better will they be utilized by bacteria. Experimentation along this line has resulted in the preparation of a product (Experimental No. 2) which, among other factors, has been hydrolized to a much greater degree than either Witte's peptone or the first experimental product. Table IV shows that where a measure of bacteriologic utilization is possible, as in production of diphtheria toxin, more than twice the potency is obtained. The results with other organisms, taking into consideration the limitations of such findings, fully substantiate the superior nutritive properties of the product.

SUMMARY.

1. Physical properties and gross chemical analyses are of secondary importance in estimating the bacteriologic availability of peptones. More valuable information is given by determination of the protein hydrolysis products present.

2. Comparative values of practical importance are best furnished by certain biologic tests permitting of quantitative estimation. These are the elaboration of diphtheria and tetanus toxins, and to a less extent the production of indol.

3. Domestic peptones furnish satisfactory tetanus toxin, but are unable to give a potent diphtheria toxin—a property possessed by Witte's peptone. Indol-producing power is shown by some of the domestic products as well as by Witte's peptone.

4. An experimental peptone is described which answers all the requirements of bacteriologic use, including the production of diphtheria and tetanus toxins, possessing superior activity.

BIBLIOGRAPHY.

[1] Bainbridge: Jour. Hyg., Cambridge, 1911, ii, 341.
[2] Park and Williams: Jour. Exper. Med., 1896, i, 164.
[3] Zipfel: Centralbl. f. Bakteriol., 1912, lxiv, 1 (Orig.) 65.
Cannon: Jour. Bacteriol., 1916, i, 536.
[4] Standard Methods of Water Analysis, A. P. H. A., 1912.
[5] Methods of Analysis, A. O. A. C.—Bull. No. 107 (Rev.) Bur. of Chem. U. S. Dept. of Agr., 1912, 4.
[6] Mohr: Cited in Treadwell-Hall, Analytical Chemistry, John Wiley & Sons, 1908, ii, 545.
[7] Bömer: Ztschr. f. anal. Chem., 1895, v, 562.
[8] Bigelow and Cook: Jour. Am. Chem. Soc., 1906, xxviii, 1496.
[9] Bovie: Jour. Med. Research, 1915, xxxiii, 295.
[10] Harding and MacLean: Jour. Biol. Chem., 1915, xx, 217.
[11] Salkowski: Cited by Ford: Proc. Am. Pub. Health Assn., 1900, xxvi, 305.
[12] Ehrlich: Cited by Böhme: Centralbl. f. Bakteriol., 1905, xl, 1 (Orig.), 129.
[13] Rosenau: Bull. No. 21, Hyg. Lab. U. S. Pub. Health Service, 1915.
[14] Rosenau and Anderson: Bull. No. 43, Hyg. Lab. U. S. Pub. Health Service, 1908.
[15] Clark: Jour. Infect. Dis., 1915, xvii, 109.

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ANTHELMINTIC TREATMENT FOR NEMATODE INFESTATIONS IN DOGS.*

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The two kinds of worms which are most important in this country as common and injurious parasites of dogs are ascarids and hookworms. There are two species of ascarids and two species of hookworms in dogs, but only one species of each appears to be common in this country. The ascarids are rather large worms, from 2 to 8 inches long, and may be white, yellow or pink in color. Hookworms are somewhat slenderer and shorter than an ordinary pin; they are usually white, though the females are not infrequently rather dark colored. Ascarids are fairly common almost anywhere in the United States and have been found in a fairly large proportion of dogs at practically every place where dogs have been examined for worms. Hookworms are most abundant in the Southern States and in localities where there is plenty of moisture and warmth; they are not very abundant in the colder and drier portions of the United States.

As a general rule, dogs in this country which are said to have "worms," have ascarids. Ascarids are sufficiently large so that the owner of the dog is apt to see them when they are passed or vomited. Older dogs usually show but few and vague symptoms of worm infestations, but pups often suffer severe reactions as a result of the presence of these worms. In a general way, worm infestation is an afebrile condition with unthriftiness, which is reflected in the poor condition of the animal's coat, and with constipation or diarrhea as a common accompaniment. At times, reflex nervous disturbances accompany these conditions; this is especially apt to be true of pups, as the more delicate mucosa of the digestive tract in young animals is not accustomed to the insults of worms, bones, hair and other irritants, as is the mucosa of older animals.

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*Read before the Southeastern Michigan Veterinary Medical Association, October 10, 1917.

Since ascarids are the worms which an owner usually has in mind when he says that a dog is "wormy," ascarids are the worms the veterinarian is usually called to treat. Fortunately, the ascarid is one kind of worm that is easy to remove. It is, of course, necessary to use suitable drugs in adequate dosage under proper conditions, but fortunately we have such drugs and they are drugs which are safe in therapeutic dosage, as well as effective.

The best drug for use in removing ascarids, as Hall and Foster (1917) have shown experimentally, is oil of chenopodium. It has a very high efficacy in single dosage, and can be depended on to remove all or nearly all of the ascarids present in the great majority of cases. No drug can be depended on to remove all worms in every instance, with a single therapeutic dose, but oil of chenopodium comes closer to accomplishing this feat than any drug of which we are aware. In our experience it does not appear to be necessary to give a purgative to a dog the day before treatment in order to secure good results. However, it is probably of some use under certain circumstances in cleaning the digestive tract and protecting the dog. In the morning, before feeding, give the dog the oil of chenopodium and at the same time give at least an ounce of castor oil to dogs of average size (10 kilos; 22 pounds). Castor oil is said to be dangerous with such drugs as oleoresin of male fern or thymol; it is the best possible protection, so far as we know, against the injurious effects from oil of chenopodium, and its simultaneous administration increases the efficacy of the chenopodium. Don't feed the dog for at least two hours after treatment. The dose of oil of chenopodium which I have arrived at experimentally is about .1 mil per kilo of weight of dog. For practical purposes the following is effective: Give 5 minims to dogs weighing 10 pounds or less; 10 minims to dogs weighing 10 to 20 pounds; 15 minims to dogs weighing 20 to 30 pounds; and not to exceed 20 minims to dogs of any weight over 30 pounds. These doses will be found adequate and represent about the minimum efficacious dose. It is always advisable to employ the minimum effective dose of almost any anthelmintic in order to minimize the injurious effect on the host. The dose for toy dogs should be cut to 2 or 3 minims if the size and condition of the animal seem to require this. Oil of chenopodium may be given in castor oil, but this method of giving it usually results in profuse salivation, a decidedly unpleasant thing about the house. It may also be given in the ordinary capsule, but this involves the danger of a capsule opening while it is being forced down a dog's throat, with a resultant pneumonia due to the irritant action of the chenopodium, a condition which may terminate in the death of the patient. In the writer's experience, the safest and most satisfactory way of administering oil of chenopodium to dogs is in the soft or elastic capsule. Every veterinarian has a preferred method of administering medicines, but an easy way to give these capsules is to have a capable assistant or the dog's owner hold the animal's jaws open, with gloved hands if necessary, while the capsules are pushed down the throat and the castor oil poured after them. If the capsules are not swallowed before the castor oil is given, they will usually go down readily with the oil, especially if the dog is given a slap on the side of the jaw as he is released. Occasionally dogs will vomit more or less after the administration of oil of chenopodium, and dogs occasionally vomit after the administration of castor oil; the dog should therefore be taken after treatment to some place where this result will occasion no damage. Even when the dog vomits, it will usually be found that an adequate anthelmintic action will follow the treatment. I find that I can give .6 mil of chenopodium per kilo of dog, with large amounts of castor oil, without killing the dog, or can repeat the doses noted here a number of days in succession, but in veterinary practice it is always advisable to stay well on the safe side and not repeat the treatment inside of a week. This is not for the reason that the dog may die; that is quite unlikely. It is simply in the interest of the patient, to allow time for the subsiding of irritation, inflammation or toxic effects in any degree that may result from the treatment. Should an individual dog show an idiosyncratic intolerance for chenopodium, give large doses of castor oil. Adrenalin and digitalis are also indicated; these counteract the lowered blood pressure and depressant effects from the chenopodium. In order to be reasonably sure that a dog is completely freed from ascarids, treatment should be repeated in the course of one or two weeks, not earlier than one week, whenever possible. This second treatment should fail to bring away worms, but in some instances the first treatment will not be successful in removing all the ascarids present. I find the treatment outlined here 100 per cent effective in nearly all cases.

Oil of chenopodium is being used in human medicine to a great extent against hookworms. It will remove hookworms from the dog, also, but not with the same certainty and efficacy that it displays against ascarids. If chenopodium is used against hookworms in dogs, it would be advisable to repeat the treatment weekly, using the same dose as for ascarids. It would be inadvisable to repeat oftener than once a week, as the digestive tract in hookworms cases is already suffering from the attacks of the worms and in no condition to stand too much additional irritation. The number of treatments should be based on consideration of the patient's condition, or on fecal findings, or on both things. Chloroform in amounts two to three times as great as the dose of oil of chenopodium is more effective against hookworm than is the chenopodium, as Hall and Foster (1917) have shown.

Santonin is another drug which may be given for removing ascarids, but it has nothing like the efficacy of oil of chenopodium when either one is given in single therapeutic doses. To secure results from santonin it is necessary to repeat the dose a number of times. It may be given, 1 grain of santonin and 1 grain of calomel a day, as often as necessary, having due regard for its effect on the patient, and especially on the kidneys, and in some cases this method might be preferred to the administration of a single dose of oil of chenopodium, but for certainty of results and saving of time, chenopodium is the preferred treatment. The treatment just outlined, 1 grain each of santonin and calomel daily over long periods, is a very effective treatment for the removal of whipworms, as only an occasional dose of anthelmintic enters the cecum, where these worms are lodged, and treatment must be repeated to ensure removal of these worms. Santonin is of no value against hookworms, even in oft repeated doses.

REFERENCE

Hall, Maurice C., and Foster, Winthrop D., 1917. Oil of chenopodium and chloroform as anthelmintics. Preliminary note. *J. Amer. Med. Ass.*, v. 68, (26), June 30, pp. 1961-1963.

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THE HYPODERMIC USE OF IRON.*

By L. W. Rowe, M.S., Detroit, Michigan.

The use of iron internally as a tonic in cases of anemia has been for years and still is a common procedure. This treatment is entirely safe, but is also somewhat unreliable, due to the incomplete absorption and assimilation of the iron. The difficulty has been only partially obviated by the internal use of complex organic preparations of iron, several of which have been placed on the market.

In recent years the hypodermic administration of iron has become increasingly popular. For this purpose the iron is usually presented in the ferric form as iron and ammonium citrate, since this is one of the least irritating and most soluble of the salts of iron.

Largely due to the comparatively complete and rapid absorption which follows the intramuscular injection of a solution of an iron salt, the toxic action of the iron is sometimes evidenced, and reports of severe systemic reactions following the hypodermic injection of iron are not rare. Meyer and Gottlieb state that the hypodermic M. L. D. of iron for dogs, cats, and rabbits is about 30 mg. per kg. body weight. The average therapeutic dose is very much smaller than this, and even then it occasionally causes trouble.

Experimental work in which iron in some form has been given by mouth to animals has been reported frequently, but very little animal experimentation has been carried out in which the iron was administered hypodermically.

It is the purpose of this short article to report the results of some experiments in which iron and ammonium citrate solution was administered hypodermically to dogs and guinea-pigs and to suggest a possible reason for the apparent inconsistency which exists between clinical evidence reported by physicians and the results obtained by animal experimentation.

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* From the Research Laboratory of Parke, Davis & Co., Detroit.

A series of experiments was carried out upon dogs to determine the effect of intramuscular injections of iron citrate solution.

Protocol No. 1.

White bulldog, weight 16 kg. Injected intramuscularly with 1 mil (Cc.) of 10-per-cent solution of iron and ammonium citrate (green) (about 1 mg. of iron per kg.) each day for four consecutive days. No noticeable symptoms were observed even as late as two weeks after the series of injections had been completed.

Protocol No. 2.

Brown bulldog, weight 17 kg. Injected intramuscularly with 2 mils of 10-per-cent solution of iron and ammonium citrate (green) (about 2 mg. per kg.) each day for four consecutive days. No evidence of any toxic action was observed. Absorption of the material injected was rapid and caused no unusual irritation.

In these experiments no untoward effects were observed following the intramuscular injection of iron citrate solution, although the larger dose is twice as great as the average human therapeutic dose.

A second series of experiments was carried out upon dogs to determine the effect of intravenous injections of iron citrate solution.

Protocol No. 1.

Brown dog, weight 10 kg. Injected intravenously with .6 mil of 10-per-cent solution iron citrate (green) (about 1 mg. of iron per kg.). No untoward symptoms observed.

Protocol No. 2.

Black and white dog, weight 7 kg. Injected intravenously with 1.5 mils of 5-per-cent solution iron citrate (Italian, green) (about 1.5 mg. of iron per kg.). No reaction observed.

Protocol No. 3.

Brown dog, weight 10 kg. Injected intravenously with 2 mils of 5-per-cent solution iron citrate (Italian) (about 1.5 mg. of iron per kg.). No reaction observed.

Protocol No. 4.

Black dog, weight 8 kg. Injected intravenously with 1.25 mils of 10-per-cent solution iron citrate (about 2.4 mg. of iron per kg.). No reaction observed.

Protocol No. 5.

Brown dog, weight 10 kg. Injected intravenously with 3 mils of 10-per-cent solution iron citrate (about 4.5 mg. of iron per kg.). No symptoms.

Protocol No. 6.

Black dog, weight 8 kg. Injected intravenously with 1.5 mils of 10-per-cent iron citrate solution (about 2.8 mg. of iron per kg.) which had been neutralized wth sodium carbonate and an excess of the carbonate added. This slightly alkaline solution did not cause any toxic symptoms.

A third series of experiments, using guinea-pigs as the test animals, had for its object the determination of the subcutaneous toxicity of iron citrate solution.

Protocol No. 7.

Guinea-pig, about 300 gm. weight, injected subcutaneously with 1 mil of 10-per-cent solution iron citrate (about 50 mg. of iron per kg.). Pig died within twelve hours.

Protocol No. 8.

Guinea-pig, about 300 gm. weight, injected subcutaneously with .5 mil of 10-per-cent solution (about 25 mg. of iron per kg.). Pig died within twenty-four hours.

Protocol No. 9.

Guinea-pig, about 300 gm. weight, injected subcutaneously with .4 mil of 10-per-cent solution (about 20 mg. of iron per kg.). Pig lived.

Protocol No. 10.

Guinea-pig, weight about 300 gm., injected subcutaneously with 1 mil of 5-per-cent solution iron citrate (Italian, green) (about 25 mg. of iron per kg.). Pig died about two days later.

The last four experiments (7 to 10 inclusive) indicate that the toxicity of iron citrate solution (green), when administered subcutaneously to guinea-pigs, is about 1.5 mils of 10-per-cent solution or 0.15 gm. of the salt per kg. body weight (about 25 mg. of iron per kg.). This compares favorably with the results reported by Meyer and Gottlieb, since their dose is based only on the amount of iron in the compound, and they used larger animals.

Schmiedeberg speaks of the marked toxicity of the double salts of iron when a solution of these is rendered weakly alkaline in reaction and injected subcutaneously or intravenously. Protocol No. 6 failed to corroborate the work of Jacoby as cited by Schmiedeberg.

The experiments reported in this article are largely negative and do not bear out the occasional unfavorable clinical reports. However, in severe cases of anemia in which iron is used as a therapeutic measure it should not be so surprising if, sometimes, a rather marked systemic reaction is observed. The dose generally used in such cases is too large to be absorbed properly and the iron entirely changed into hemoglobin. The experiments carried out upon healthy dogs represent a much more vigorous therapeutic treatment than that from which some human patients have been reported to have suffered severe reactions. There is no mention made in any of the standard works on pharmacology that dogs are unusually resistant to the toxic action of iron, so that the difference between the results on animals and those reported by physicians must be accounted for in some other way. The most logical explanation of this difference seems to be that the anemic patient, due to his lowered vitality, is unable to assimilate large doses of soluble iron salts and systemic disturbances result.

The local irritation produced by the hypodermic use of iron solution is frequently encountered. In experiments upon healthy animals the local irritation produced has not been found to be sufficient to merit serious consideration. However, as in the case of other heavy metals, iron apparently causes considerable local irritation when injected hypodermically into anemic patients. This difficulty will probably never be entirely overcome until a satisfactory colloidal solution of iron is prepared for hypodermic administration.

Ferric ammonium citrate when properly prepared can usually be safely used hypodermically in a dose of 0.1 gm. (1 mil of a 10-per-cent solution). Sollmann makes a similar statement with regard to *ferric citrate*. However, in severe cases a smaller dose of a less concentrated solution is apparently better suited for hypodermic administration if an undesirable systemic reaction is to be avoided.

The conclusions to be drawn from the experimental data submitted and from the small amount of available literature on the subject are:

First, that animal experimentation does not corroborate the untoward clinical results that are frequently obtained in the hypodermic use of soluble iron salts; and,

Second, that the most logical explanation of these untoward clinical results seems to be that the weakened patient is unable to assimilate the iron properly and symptoms of poisoning consequently occur.

BIBLIOGRAPHY.

Meyer and Gottlieb: Pharmacology, page 443 of Halsey's translation.
Schmiedeberg: Pharmacology, page 554 of 7th Ed.
Jacoby: Dissertation, 1887.
Sollmann: Pharmacology, page 629.
Cushny: Pharmacology.
Macallum: *Jour. of Phys.*, vol. 16, 268.
Müller: *Virchow's Archiv*, vol. 164, 436.

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THE VALUE OF THE YEAST VITAMINE FRACTION AS A SUPPLEMENT TO A RICE DIET.

By A. D. Emmett and L. H. McKim.

(From the Research Laboratory of Parke, Davis & Company, Detroit.)

(Received for publication, September 28, 1917.)

In the present paper, the authors report the first of a series of investigations that has to deal more particularly with the efficiency of the vitamines from autolyzed brewer's yeast, in respect to their value as an *adjuvant* to a diet that has been shown to be deficient in some particular vitamine. Thus, while it is known from the work of Chamberlain, Vedder, Funk, Suzuki, Eykmann, Williams, Seidell and others, that polished rice as the sole article of diet, in the case of pigeons and man, will produce typical polyneuritis and beri-beri, respectively, and that these dietary deficiency diseases can be treated effectively by giving a dose of the vitamine fraction from brewer's yeast, *it is also pertinent to know how effective a rational treatment with this same vitamine will be as a therapeutic agent in convalescent subjects.* Stating this in another way, using the same illustration, will the supplementing of the polished rice diet with the yeast vitamine (where pigeons have been cured of polyneuritis) not only prevent the recurrence of the disease, *but will it bring about* as full a recovery, measured by the gain in body weight, as a diet that is known to be complete in respect to the particular vitamines involved?

Hopkins, Stepp, Funk and coworkers, Osborne and Mendel, McCollum and coworkers, Drummond (1), and others have given abundant evidence of the importance of accessories in the normal dietary. Funk (2) found when polished rice was supplemented with a crystalline vitamine prepared from yeast that it was a complete food in the sense that the diet not only prevented the onset of polyneuritis but brought about a gain in weight. These tests extended over only 29 days. He concluded that there was no justification for the belief that there might be two vitamines—one for curing polyneuritis, and one for maintaining weight. Funk, Lyle, and McCaskey (3) report a study where they endeavored to determine the nutritive value of yeast, polished rice, and white bread from the standpoint of the influence of vitamines upon the metabolism of man. It was found that the value of dried yeast as a source of protein was not very good. The high content of the purines in the yeast caused a noticeable rise in uric acid in the blood, and showed definitely that yeast should not be used too freely as a substitute for other foods. Further, when the vitamine- and purine-free diet was supplemented with a vitamine preparation, made by Seidell's method (4), there seemed to have been no apparent effect produced on the nitrogen balance. The authors state that this may have been due to the shortness of the metabolism period or to the fact that the vitamine preparation was not sufficiently active. They found that a vitamine-free diet of white bread and polished rice required more nitrogen than a diet of whole wheat bread or potato to produce a positive nitrogen balance.

In the particular case of polished rice, McCollum and Davis (1) have shown, in feeding young rats, that it is necessary for normal growth to supplement the diet with both the water-soluble and fat-soluble accessories or vitamines, even though the protein, carbohydrate, and salts in the ration are liberal in amount. McCollum and Kennedy (5) report in their work on polyneuritis in pigeons that the water-soluble accessory is directly concerned in the prevention and cure of this disease and that the fat-soluble fraction is related to maintenance.

Funk and Cooper both demonstrated that autolyzing yeast increased its value as a curative agent for polyneuritis. Seidell (6) has confirmed this more recently. Funk and Macallum (7) believe that autolyzed yeast contains at least two vitamines—one which will cure polyneuritis and the other which will stimulate growth. They found that the addition of this yeast, along with butter fat, to a ration accelerated growth in rats; that when lard was introduced in place of butter fat no growth resulted if the yeast was dried, but if the yeast was moist, growth resulted, although to a lesser degree than with the butter fat; and it seemed that the growth-promoting substance in yeast was practically identical with the polyneuritic vitamine, except that it required more of the former to bring about growth than of the latter to produce a cure.

Seidell fed pigeons a diet of polished rice and added to it an autolyzed yeast vitamine preparation, made in accordance with his method, using Lloyd's anhydrous aluminum silicate as the adsorptive agent. He found with this supplement that normal pigeons when put upon this diet from the first showed no bad effects, but in some cases they made slow but gradual gains in weight over a period of 60 days. Williams and Seidell (8) were able to separate a crystalline product from the Seidell yeast vitamine that contained an active principle. This material was curative, and in administering it to polyneuritic pigeons it brought about gains in weight on a polished rice diet. The gains amounted to about 80 per cent of the initial weight in tests covering 80 days. However, these gains took place during the first 25 to 30 days following the treatment, after which the weight remained practically constant. In one case, a polyneuritic pigeon regained its original weight in 30 days, but an unusually large dose (50 mg.) of the crystalline material was given. In another report, Seidell showed that the autolyzed yeast filtrate will stimulate an appreciable gain in weight in the case of a normal pigeon if given in sufficiently large amounts—much more than that needed for curing pigeons of polyneuritis. He estimated that the diet of pigeons should contain a little less than 0.0033 per cent of vitamine.

Funk (9) in considering the dietary factors in relation to growth states that while yeast promotes growth, the Seidell vitamine preparation from autolyzed yeast with Lloyd's reagent was greatly deficient in the growth stimulant and has lost some of its antiscorbutic property.

EXPERIMENTAL.

The pigeons, with the exception of a few which were kept for normal controls, were put upon polished rice as the sole food, along with distilled water. At first, the birds on the polished rice were forcibly fed after they began to show a gradual loss of appetite. Later, this procedure was discontinued, as it appeared in comparison with the pigeons which were not forcibly fed that the additional labor involved was possibly not justified. We are not fully convinced, however, at this time, that this is true in all cases, inasmuch as the factor of inanition in some cases seemed to introduce complications which rendered some pigeons more susceptible to infection.

When the pigeons came down with typical polyneuritis and reached the critical stage, they were treated with the Seidell autolyzed yeast vitamine. Special care was used to keep the birds, during this period of the disease and treatment, isolated in separate cages, as it was evident to us that absolute quiet was a factor which tended to hasten the recovery of the pigeons. For 24 hours therefore after treatment, the pigeons were handled only when absolutely necessary, given water *ad libitum*, and only a very limited amount of feed.

The diet, after the treatment, consisted of one of the following: polished rice with vitamine, shelled corn, brown or natural rice, brown rice with vitamine, barley, unhulled oats, and hulled oats. In general, one of these diets was fed until the pigeons seemed to cease to gain in weight, when another diet was substituted for it. No attempt was made to adjust the mineral deficiencies in the different foods.

Preparation of the Yeast Vitamine.—In general, the method of Seidell was followed in separating the vitamine from the autolyzed brewer's yeast filtrate. We introduced certain modifications relating to the removal of the liquor from the yeast, the drying of the yeast, the length of time of autolyzing, etc.

Several forms of aluminum silicate were tested as to their adsorptive power for vitamine, among them being infusorial earth; the trade products "filtercel" and "celite B;" the usual form of fullers' earth; and the special form of fullers' earth, called Lloyd's reagent. Lloyd's reagent was taken as the standard. The activated vitamine materials were made into tablets, a definite amount of lactose being added so as to provide the necessary excipient to give them the proper body.

DISCUSSION.

Effect of the Reagents in Producing Polyneuritis.—In Chart 1 four typical curves are shown representing the effect of feeding pigeons polished rice supplemented with one of the following reagents: Lloyd's special fullers' earth, ordinary fullers' earth, and lactose. The results show conclusively that these reagents had no influence in retarding or hastening the onset of the polyneuritic attack or in effecting the loss in weight. The difference in the length of time that was required to bring about the typical stage of polyneuritis was no greater than the variations in time that we have found with a large number of pigeons that we have fed. The time of the onset depends in part, it seems, upon the thriftiness of the pigeons at the start, the age, the temperature of the room, and whether the birds are forcibly fed or not.

Comparative Value of the Lloyd's Reagent and Fullers' Earth Vitamine Preparations.—In Chart 2, Pigeons 18 and 23 represent two groups of birds that were fed polished rice alone. No. 23 was treated for polyneuritis with Lloyd's reagent yeast vitamine (L.R.) and No. 18 with the fullers' earth preparation (F.E.). The curves illustrate very clearly the comparative value of the two vitamine preparations, showing that they were apparently of equal strength, when given in the same amounts. A number of similar tests indicated definitely that one preparation was as good as the other and that both were as effective in the treatment of pigeons as the autolyzed yeast filtrate itself.

After treatment, these respective birds were placed on the polished rice diet, supplemented with the *same* kind of vitamine preparation that was used in bringing about the recovery from the attack of polyneuritis. Later, the pigeons were put on barley without vitamine, then on oats, and finally on hulled oats. Throughout this whole cycle, the two respective curves followed in almost the same direction.

Influence of the Different Dietary Treatments on Polyneuritic Pigeons.—The effect of feeding the pigeons, after they were brought down to the typical and fulminating stage of polyneuritis and then treated with the vitamine preparation, is shown in Charts 3 and 4.

Other instances of the effect upon the weight curves of feeding pigeons polished rice along with the Seidell autolyzed yeast vitamine (F.E.), are shown in the case of Nos. 37 and 52, Chart 1; and also of Nos. 10 and 15, after their recovery from the second attack of polyneuritis, Chart 4.

It was assumed that the quantity of yeast vitamine added in supplementing the rice diets ought not to be more than the amount used in the treatment for the typical attack of polyneuritis, since this amount was always as great as the quantity contained in an equal weight of brown or natural rice. It seemed to us that if a much larger amount of this particular vitamine was needed to bring about as good a recovery as could be obtained by feeding a diet of brown rice or other vitamine-containing grains, then we were dealing here with a vitamine preparation that was *qualitatively* different from that which was removed in the polishing of the rice, the difference being due either to the vitamine contained in the autolyzed yeast filtrate or to the alteration of this vitamine by the method which was used in separating it from the yeast filtrate. The amount used was equivalent to 0.03 to 0.06 gm. of the Seidell vitamine per day.

To summarize, after making allowance for the influence of confinement and environment upon the weight of the pigeons, the effect of the dietary treatments, *after recovery from a typical attack of polyneuritis,* was as follows:

(*a*) Polished rice plus the autolyzed yeast vitamine of Seidell prevented a recurrence of polyneuritis, and brought about a partial gain in weight, which after 75 to 90 days from date of recovery began to decrease and then came to a more or less constant level. The birds were active and thrifty throughout.

(*b*) Brown rice, as the sole diet, brought about very good gains.

In some cases the pigeons not only recovered all that they lost in coming down with polyneuritis, but went beyond their initial weight at the time of being put in the cage. It is barely possible, although doubtful in the light of our present knowledge, that some of the differences between the effect of this diet and the preceding one (*a*) may have been due in part to the removal of phosphorus in the polishing of the rice. This point is being studied.

(*c*) Brown rice plus the yeast vitamine produced the best gains of any diet tried. The yeast vitamine acted as a stimulant here, even though there was an ample amount of vitamine in the brown rice alone to produce normal gains.

(*d*) Shelled corn was an efficient diet, producing gains that were greater than those obtained with the polished rice plus the vitamine, but less than those with the brown rice alone.

(*e*) Barley brought about fair gains, better than the polished rice plus vitamine, but its effect was soon lost, the pigeons declining rapidly in weight.

(*f*) Unhulled oats proved to be unsatisfactory as a normal diet for pigeons.

(*g*) Hulled oats were much better than the unhulled.

CONCLUSIONS.

From these studies, which have extended over a much longer period of time than any of those previously reported on this subject, it appears:

1. That ordinary fullers' earth, ground to a definite fineness, adsorbs the yeast vitamine fraction from the autolyzed yeast filtrate as readily as the special form of fullers' earth, called Lloyd's reagent. Further, the *Kieselguhrs* (infusorial earth, "celite B," and "filtercel") do not adsorb the yeast vitamine.

2. That these two forms of activated fullers' earth are equally potent and very efficient as therapeutic agents in the treatment for typical attacks of avian polyneuritis.

3. That silicates in the form of ordinary fullers' earth, Lloyd's reagent, or infusorial earth, when added to a polished rice diet, do not inhibit or accelerate the onset of avian polyneuritis; and that lactose, used in making the vitamine tablets, is also inert in this respect.

4. That (*a*) the activated fullers' earth when given as a *rational* supplement to a polished or a brown rice diet, acts as a partial stimulant to increase the weight of the treated polyneuritic pigeons; (*b*) it does not, however, in the case of the polished rice, accelerate the increase in weight to anything like that which is produced under similar conditions with brown rice alone, corn, barley, or hulled oats; and (*c*) comparatively speaking, this activated yeast vitamine is not a complete supplement to a polished rice diet.

5. That there are apparently two so-called vitamines associated with rice polishing, one which cures polyneuritis and one which produces weight, and of these two, the Seidell yeast vitamine preparation contains chiefly the curative fraction, along with a small per cent of the other.

6. Finally, it is evident that while this activated yeast vitamine product is a valuable adjuvant to the diet in the case of convalescents from avian polyneuritis, yet, for the best results, the diet should, in addition, be made up in part at least of vitamine-containing foods, not for the purpose of preventing the recurrence of typical attacks of this dietary deficiency disease (for the preparation is able to do this) but with the object of bringing about normal gains in weight and *complete* recovery.

BIBLIOGRAPHY.

1. Hopkins, F. G., *J. Physiol.*, 1912, xliv, 425. Stepp, W., *Z. physiol. Chem.*, 1913, lxxxiv, 359. Osborne, T. B., and Mendel, L. B., *Biochem. J.*, 1916, x, 534. Funk, C., and Macallum, A.B., *J. Biol. Chem.*, 1915, xxiii, 413; 1916, xxvii, 51, 63; *Z. physiol. Chem.*, 1914, xcii, 13. McCollum, E. V., and Davis, M., *J. Biol. Chem.*, 1915, xxiii, 181. McCollum, E. V., Simmonds, N., and Pitz, W., *ibid.*, 1916, xxv, 105; xxvii, 33. Drummond, J. C., *Biochem. J.*, 1916, x, 77.
2. Funk, C., *J. Physiol.*, 1912, xliv, 50.
3. Funk, C., Lyle, W. G., and McCaskey, D., *J. Biol. Chem.*, 1916, xxvii, 173.
4. Seidell, A., *U. S. Public Health Report,* 1916, xxxi, 364; *U. S. Patent Office, No. 1,* 173, 317 (Feb. 29, 1916).
5. McCollum, E. V., and Kennedy, C., *J. Biol. Chem.*, 1916, xxiv, 491.
6. Seidell, *J. Biol. Chem.*, 1917, xxix, 145.
7. Funk and Macallum, *J. Biol. Chem.*, 1916, xxvii, 63.
8. Williams, R. R., and Seidell, A., *J. Biol. Chem.*, 1916, xxvi, 431.
9. Funk, *J. Biol. Chem.*, 1916, xxvii, 1.

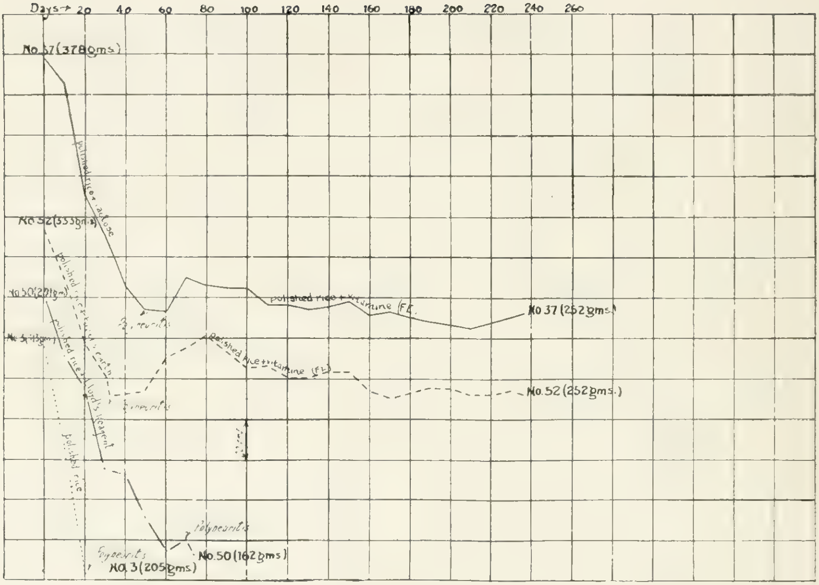

Chart 1. The curves show that the supplementing of the polished rice diet with lactose, or fullers' earth, or the special form of fullers' earth called Lloyd's reagent, has no effect upon accelerating or retarding the onset of polyneuritis. After the 55th day, the curves for Pigeons 37 and 52 illustrate the influence of feeding convalescent polyneuritic pigeons upon polished rice and yeast vitamine (Seidell).

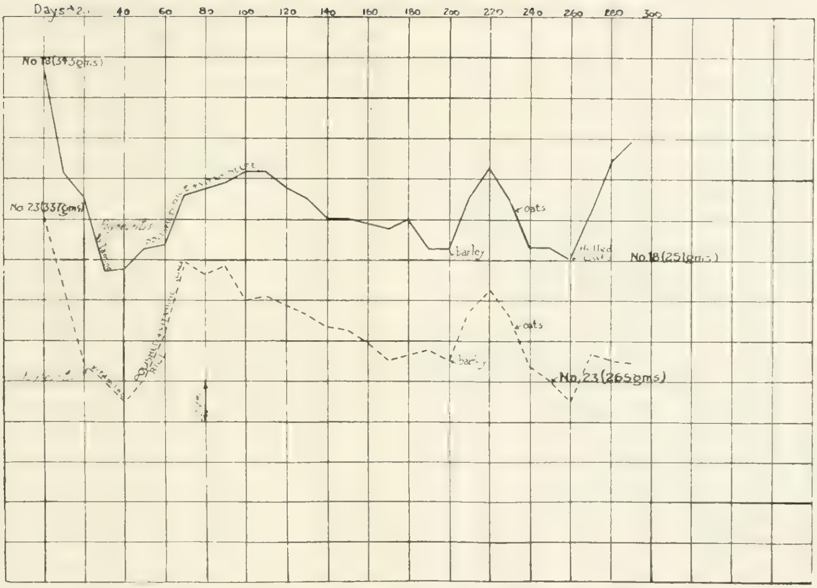

CHART 2. The two weight curves indicate how closely the vitamine preparations, made with the usual fullers' earth and the special form, Lloyd's reagent, compare as to their curative and therapeutic value. They also represent the comparative value of the rations of polished rice with vitamine, barley alone, oats alone, and hulled oats alone.

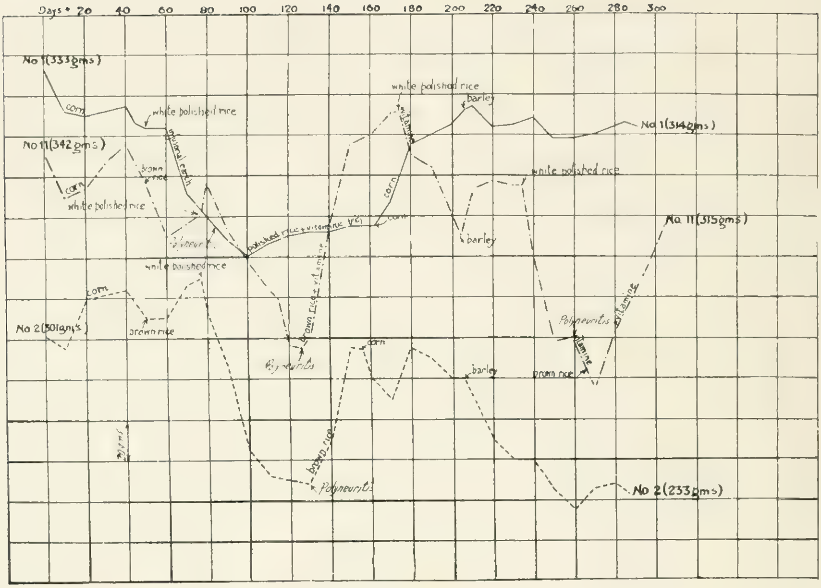

Chart 3. These weight curves show, first, the effect of confinement in the cage and environment over the first 55 to 80 days; second, the neutral effect of infusorial earth (Pigeon 1); third, the therapeutic value of yeast vitamine as a supplement to the diets—polished rice alone, polished rice with yeast vitamine, brown or natural rice with yeast vitamine, compared with brown rice alone, corn alone, and barley alone.

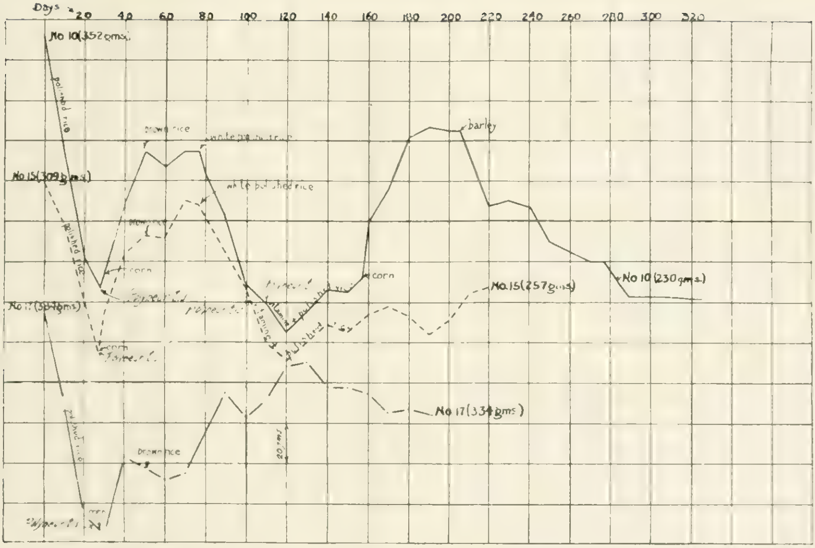

CHART 4. The curves show, first, the typical decline in weight with a polished rice diet; second, the comparative nutritive value of brown or natural rice and corn when fed to pigeons after vitamine treatment for typical polyneuritis; and third, the therapeutic value of supplementing a vitamine-free diet (polished rice) with yeast vitamine compared with vitamine-containing foods, brown rice, corn, and barley.

Studies from the Research Laboratory
Parke, Davis & Co.
Reprint No. 142, 1917

NEW SPECIES AND VARIETIES FROM MICHIGAN.*

By Oliver Atkins Farwell.

CYPERACEÆ.

Scirpus occidentalis (Watson) Chase, variety congestus, N. var.

Spikelets aggregated into a sessile glomerule, or if peduncles are present these are so short that they are hidden by the spikelets. Marl lake, No. 4294, July 9, 1916, and No. 4376, August 13, 1916. Bears the same relation to the species that variety *confertus* does to *S. rubrotinctus.* Not so common as the species.

Carex canescens Lin. var. heterostachya, N. var.

In this form of the species the terminal spike is entirely staminate. Collected on the Keweenaw Peninsula many years ago.

JUNCACEÆ.

Juncus bufonius Lin. var. ranarius, N. Var.

Juncus ranarius Song. et Perrier in Billot Annot. Fl. France et Allem. 192, 1859 not Nees, 1847. (?)

Sheathes of the lowest leaves dark red, the petals acute not awl pointed, about the length of the capsule and shorter by about a millimeter than the awl pointed sepals. In this plant the flowers are single and remote as in the species, but the petals are shorter than the sepals and not awl pointed, resembling in these characters the var. *halophilus.* River Rouge, No. 4342, July 21, 1916.

ORCHIDACEÆ.

Corallorhiza maculata Raf. var. intermedia, N. var.

Whole plant purplish yellow; lip white with two or three large, very pale purplish spots; no spots on the other petals or sepals. Copper Harbor, Keweenaw Peninsula, No. 4003, July 8, 1915. Exactly intermediate between the species and the var. *flavida*

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*19th Mich. Acad. Sci. Rept., 1917.

(Peck) Cockrl. in color and in spots which are found only on the lip and are fewer, larger, and paler than in the species. The species is common; and the var. *flavida* also is found at Copper Harbor, No. 4002, July 8, 1915.

CRUCIFERACEÆ.

ARABIS LÆVIGATA (Muhl.) Poir. var. HETEROPHYLA, N. var.

Arabis heterophylla Nutt.; T. & G. Fl. N. Amer. I, 81, 1838. (?)

Lower part of stem, including leaves, and the rosette leaves pubescent with short, spreading hairs, elsewhere glabrous and glaucous. Rosette leaves and lowermost stem leaves lyrately pinnatifid gradually passing to obovate and lacinate, thence to ovate, ovate-, oblong-, and linear-lanceolate and dentate or entire. The rosette leaves and those on the lower part of the stem are purplish underneath. Flowers erect on erect or slightly spreading pedicels; petals white, six or seven millimeters long, from one-half as long again to twice as long as the sepals; pods spreading or recurved five to seven centimeters long by one and three-quarters to two millimeters wide with a short, stout style not over a millimeter long or sessile stigma. Zoological Park near Royal Oak No. 4210, July 13, 1916.

This plant cannot be typical *A. lævigata* as that is always described as entirely glabrous and glaucous, while the lower parts of this plant are pubescent. The longest leaves are on the basal part of the stem and are about five centimeters long; the shortest are the uppermost and are six or seven millimeters. It may be the *A. heterophylla* of Nuttall which was described as with hirsute radical leaves.

SAXIFRAGACEÆ.

TIARELLA CORDIFOLIA Lin. var. BRACTEATA, N. var.

Scape bearing on the middle section a petioled leaf somewhat similar to the basal but much reduced in size; petiole three millimeters to two centimeters long and the ovate, three-lobed blade from one-half to two centimeters in length and breadth. Near Disco, Macomb Co., No. 4153, May 25, 1916.

CALCARATACEÆ.

VIOLA CANADENSIS Lin. var. PUBENS, N. var.

More or less densely and almost canescently pubescent throughout with short, spreading hairs. Otherwise like the specific type which is here considered to be the common glabrous form. Near Disco in Macomb Co., No. 4155, May 25, 1916.

VIOLA CONSPERSA Reichb. var. MASONII, N. var.

Flowers pure white. Has the appearance of V. striata but can be readily distinguished by the entire sepals. Near Utica, No. 4163, June 6, 1916. Named for Mr. E. W. Mason of Lapeer, who first observed it near Utica and who called my attention to it.

VIOLA ROSTRATA Ait. var. ELONGATA, N. var.

Spur 15 to 18 millimeters long; sepals narrow, one millimeter or less wide. Near Utica, No. 4166, June 16, 1916. Differs from the species in having a much longer spur and much narrower sepals.

LABIATACEÆ.

SCUTELLARIA LATERIFLORA Lin. var. ALBIFLORA, N. var.

Flowers white. Grosse Isle, No. 4385, August 20, 1916.

RINGENTACEÆ.

MIMULUS ALATUS Ait. var. CHANDLERI, N. var.

Flowers pale lavender to white. Collected by Mr. B. F. Chandler of Detroit, at Gawkler's Point in August, 1916.

MIMULUS RINGENS Lin. var. CONGESTA, N. var.

Peduncles shorter than their subtending leaves. Grosse Isle, No. 4391, August 20, 1916. In the specific type the peduncles are longer than their subtending leaves on the upper parts of the plant, forming a broad and open infloresecence. In this variety the peduncles are much shorter, forming a narrow, close inflorescence.

Veronica Anagallis-aquatica Lin. var. glandulosa, N. var.

Stem more or less glandular pubescent. Whole plant smaller and less succulent than in the specific type. Zoo Park, near Royal Oak, No. 4323, July 13, 1916.

Pedicularis lanceolata Mx. var. hirsuta, N. var.

Stems and branches hirsute. In the typical plant the stems and branches are glabrous. On Parkedale Farm, No. 4418½, September 3, 1916; No. 4016, September 3, 1915; No. 4134, October 15, 1915, from Detroit.

COMPOSITACEÆ.

Aster Novæ-Angliæ Lin. var. monocephala, N. var.

Stem, simple, strict, ending in a single head. Belle Isle and other places in Detroit.

Heliopsis scabra Dun. var. intermedia, N. var.

Stems glabrous; leaves firm and harshly scabrous as in the species. Near Rochester, No. 4330, July 15, 1916, and No. 4349, August 12, 1916, near Farmington. The species has a very scabrous stem as well as leaves.

Lactuca Canadensis Lin. var. albocærulea, N. var.

Flowers pale blue to white. Bloomfield, No. 4394, August 22, 1916.

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Studies from the Research Laboratory
Parke, Davis & Co.
Reprint No. 143, 1917.

RARE OR INTERESTING PLANTS IN MICHIGAN.*

By O. A. Farwell.

POLYPODIACEÆ.

Filix cristata (Lin.) Farwell var. Clintoniana (D. C. E.) Farwell.

A rare fern. Only three stations are given for it by Beal in the Michigan Flora. It is to be found in cool, moist woods near Utica. No. 4179, June 6, 1916.

SPARGANIACEÆ.

Sparganium simplex Hudson.

Several species have been segregated from the S. simplex of our earlier manuals. I have not seen anything answering to the *S. simplex* Hudson as now understood. *S. Americanum* Nutt. and *S. diversifolium* Græbner should replace the S. simplex of Beal's Michigan Flora.

Sparganium Americanum, Nutt.

4248, June 29, '16, Cass Lake; 812, Aug. 25, '90, Keweenaw County; 812a, July 24, '10, Rochester.

Sparganium diversifolium Græbner.

Keweenaw County, No. 219, June 16, 1885; Borders of Cass Lake, No. 4249, June 29, 1916.

Sparganium diversifolium var. acaule (Beebe) F. & E.

2106, Aug. 15, '09, Rochester; 219a, June 16, '85, Keweenaw County.

FLUVIALACEÆ.

Potamogeton Americanus C. & S.

Said to be a rare species in Michigan. It is to be found in a slip at the Great Engineering Works, at River Rouge. No. 4340, July 21, 1916.

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*19th Mich. Acad. Sci. Rept., 1917.

JUNCAGINACEÆ.

TRIGLOCHIN MARITIMUM Lin. Sp. Pl. I, 339, 1753.

Triglochin maritimum Lin. var. *exangulare* Reichenb. Ic. Fl. Germ. VII, 28, t. LII fig. 93, 1845.

TRIGLOCHIN MARITIMUM Lin. var. SEXANGULARE Reichenb. l.c. fig. 92.

Triglochin elatum Nutt. Gen. I, 237, 1818.

Triglochin maritimum Lin. var. *elatum* (Nutt.) A. Gr. Man. 437, 1856.

There are two forms of the Spike Grass; one with ovoid fruit without a neck at the apex and with rounded carpels; the other has oblong fruit contracted into a neck at the apex and the carpels have depressed backs. The former is typical of the species and the edges of the carpels are not sharply acute; in the latter which is characteristic of the variety the carpels are sharply acute or winged. Reichenbach's varietal name is older by seven years than that of Gray and should be adopted. Both the species and the variety were accredited to Michigan (Great Lakes region), but all the Michigan material which has passed under my observation is of the variety. The cross section of the fruit of the species is circular in outline, while that of the variety is strongly and regularly angular with concave edges between the angles. Marl Lake, No. 4292, July 9, 1916.

ALISMATACEÆ.

SAGITTARIA LATIFOLIA Willd.

In most of its forms this species is common throughout the state. At Marl Lake a form was found that had narrowly linear blades and lobes which unquestionably is the *forma gracilis* (Ph.) Robinson. Not before reported from the State. No. 4362, Aug. 13, 1916.

GRAMINACEÆ.

ZIZANIA AQUATICA Lin.

There are two species of Water Oats or Indian Rice in Michigan. One is a tall, coarse plant with broad leaves and is the *Z. palustris* Lin. The other is a slender, small plant with very narrow leaves and is the *Z. aquatica* Lin. They are seldom found growing together, but when they are so found they are readily distinguished, and do not seem to intergrade. Marl Lake, No. 4372, Aug. 13, 1916, and No. 2115 from Huron River near Geddes, Aug. 21, 1909, are *Z. aquatica* Lin.; No. 1297, August 12, 1892, from Belle Isle; No. 1297a, August 14, 1909, from Grosse Isle; and No. 1297b, from Huron River near Geddes are *Z. palustris* Lin.

SPARTINA PATENS (Ait.) Muhl. var. JUNCEA (Mx.) A. S. H.

An Atlantic salt-water-marsh plant that has become established in the salt marshes at Oakwood. No. 4111, Sept. 23, 1915.

CYPERACEÆ.

TRICHOPHYLLUM ROSTELLATUM (Torr.) Farwell.

Said to be rare in Michigan. The tough, sterile stems recline upon the earth, and when well rooted at the proliferous apex are very liable to trip up the unwary traveler. Marl Lake, No. 4302, July 9, 1916.

ERIOPHORUM CYPERINUM Lin. var. PELIUM (Fernald), N. Comb.

Scirpus cyperinus (Lin.) Kunth var. *pelius* Fernald, Rhodora VIII, 164, 1906.

In swamps throughout the State; infrequent in southern Michigan but common in the Upper Peninsula. Marl Lake, No. 4358, August 13, 1916.

TRIODON ALBUS (Lin.), N. comb.

Schœnus albus Lin. Sp. Pl. 44, 1753.

TRIODON ALBUS (Lin.) Farwell var. MACER (Clarke), N. comb.

Rhynchospora alba (Lin.) Vahl. var. *macra* Clarke in Britt. Trans. N. Y. Acad. Sci. XI, 88, 1892.

The variety *macer* has not heretofore been reported from Michigan, in so far as I am aware. It is a tall, coarse plant with a corymb 2-4 cm. broad. Marl Lake, No. 4375, August 13, 1916. Bloomfield Center, No. 4399, August 22, 1916.

MARISCUS MARISCOIDES (Torrey) O. K. Said to be infrequent. Marl Lake, No. 4303, July 9, 1916, and No. 4364, August 13, 1916.

SCLERIA VERTICILLATA Muhl.

A rare species in Michigan. I have seen it only on marl beds. Marl Lake, No. 4367, August 13, 1916.

CAREX DIANDRA Schrank var. RAMOSA (Boott) Fernald.

Apparently this form is more common in Michigan than the species, at least in the southeastern section. Marl Lake, No. 4312, July 9, 1916.

CAREX CHORDORHIZA Lin. f.

This species, recorded as rare in Michigan, is quite common in an extensive peat bog in the vicinity of Pontiac. No. 4223, June 18, 1916.

CAREX POLYGAMA Schk. var. HETEROSTACHYA (Anderss.), N. Comb.

Carex Buxbaumii Wahl. var. *heterostachya* Anderss. Cyper. Scand. 39, 1849.

In this form the terminal spike is entirely staminate. Quite common in places. Algonac, No. 4230, June 22, 1916; Detroit, No. 2034, July 14, 1907.

CAREX DEFLEXA Hornem.

While on a botanical excursion through the fields in the vicinity of Algonac on June 22, 1916, in search of some early flowering Bartonia Virginica that had been found there the preceding autumn, a small area of ground was discovered which was fairly well covered with a small, dark green Carex that on close examination proved to my great surprise and delight to be the rare *Carex deflexa.* It was more than a score of years since I had discovered this species on the Keweenaw Peninsula and it had not been seen in the interim, and, so far as I am aware, it had not been reported by others, so that these two places are the only known localities for the species in Michigan. The field was low, moist ground only a few feet above the level of Lake St. Clair. The Carex was growing in moss that covered the low knolls or elevations as well as the hollows. The typical form, No. 4226½, is a low plant with leaves not over 20 cm. long and erect, the culms being not over one-half that length and strongly curved. Another and more common form, No. 4226, is the variety *Deanei* Bailey, which is more lax and has much longer leaves equaled or exceeded by the culms, which are more slender and less curved than in the species. The station near Algonac is close to the southern limits of the range of the species.

CAREX LIMOSA Lin.

This is a very slender sedge that may be looked for in peat bogs. I have not found it in other habitats. It cannot be classed as a common plant in Michigan. Bloomfield, No. 4193, June 15, 1916.

CAREX LAXICULMIS Schw.

A rare sedge in moist woods. The large patches of broad, glaucous, basal leaves at once suggest *C. granularis* or its variety *Haleana*, but the culms and spikes readily show it to be not that species. Vicinity of Palmer Park, No. 4279, July 2, 1916.

CAREX PSEUDO-CYPERUS Lin.

A rare sedge in Michigan. I first made its acquaintance last year, 1916, at Cass Lake, where it was not at all plentiful. No. 4247, June 19.

CAREX PSEUDO-CYPERUS Lin. var. COMOSA Boott.

Larger and coarser than the species with thicker spikes more loosely flowered and perigynia with a longer beak, the teeth of which are more spreading; the perigynia usually are horizontally spreading. Generally considered as a distinct species under the name of *Carex comosa* Boott. There are intermediate forms, however, with the perigynia strongly reflexed, and it might better be considered as a variety of *C. Pseudo-Cyperus* than as a separate species. Cass Lake, No. 4246, July 29, 1916; Tacoma, No. 4266, July 2, 1916.

JUNCACEÆ.

JUNCUS PELOCARPUS, E. Meyer.

One of the rarer species in Michigan. May be proliferous. Marl Lake, No. 4365, August 13, 1916.

LILIACEÆ.

QUAMASIA ESCULENTA (Nutt.) Raf.

Frequent on the banks of the Huron near Rockwood, No. 4149, May 21, 1916. Nuttall was the first to discover and name this species. The original habitat or type locality is "near the confluence of Huron River and Lake Erie;" the station at Rockwood, the only one at present known which might come within the limits of the type locality, may be the identical place where Nuttall originally found the species. Rafinesque established the genus Quamasia in 1818, naming one species, *Q. esculenta,* which by many present-day botanists is considered to be the western *Q. Quamash* (Pursh.) Coville. I have not seen Rafinesque's publication, but in his Medical Flora II, 255, 1830, his concept of his *Quamasia esculenta* is exactly that of Nuttall's, *i.e.*, it includes both the eastern *Camassia Fraseri* Torr. and the western *C. esculenta* Lindley. If the above is the proper interpretation to be applied to Rafinesque's *Q. esculenta,* then it has been erroneously referred to the western species. Nuttall's species (Fraser's Catalogue, 1813, and Genera I, 219, 1818) was based on plants collected by Nuttall himself in 1810 at the type locality above cited; he also included the western plant under the impression that it was the same species. Rafinesque's name, if based upon Nuttall's, as is probably the case, must of course belong to the eastern species.

ALETRIS FARINOSA Lin.

A rare plant in Michigan. Prefers dry, sandy soil. Vicinity of Marl Lake, No. 4309, July 9, 1916.

SMILAX ROTUNDIFOLIA Lin. var. CADUCA (Lin.) A. Gr. in Darl. Fl. Cest. 319, 1853.

S. caduca Lin. Sp. 1030, 1753.

S. quadrangularis Muhl. in Willd. Sp. Pl. IV 775, 1806.

S. rotundifolia Lin. var. *quadrangularis* (Muhl.) Darby, Bot. So. States 518, 1855.

Usually rather low and often with very stout, flat spines, colored at the tips, Algonac, No. 3156, June 22, 1916.

SALICACEÆ.

SALIX FRAGILIS Lin.

River banks at Stoney Creek. An escape from cultivation. Seems to have taken the place of S. alba in this vicinity. No. 4152½, May 25, 1916.

SALIX PEDICELLARIS Pursh. var. HYPERGLAUCA Fernald.

A very attractive bog willow. Frequent in bogs and wet meadows. Has passed in our manuals for the European *S. myrtilloides*. Bloomfield, No. 4198, June 15, 1916.

ARISTOLOCHIACEÆ.

Asarum reflexum Bicknell var. ambiguum Bicknell.

Woods in Macomb Co., near Disco, No. 4156, May 25, 1916.

A. Canadense Lin. is credited to Michigan, but such plants as I have seen are referable to this species or to *A. acuminatum* (Ash) Bicknell.

PERSICARIACEÆ.

Polygonum dumetorum Lin.

This species probably has been confused with *P. scandens* Lin. and is more frequent in the state than has been supposed. Rochester, No. 4417, Sept. 3, 1916.

ALSINACEÆ.

Scleranthus annuus Lin.

Fields at Detroit and Wyandotte. Reported by Dr. McCall of the latter place as overrunning cultivated fields. No. 4314, July 11, 1916.

CRUCIFERACEÆ.

Berteroa incana (Lin.) DC.

A hoary pubescent, branching herb that is rapidly spreading and bids fair to rival the Wild Mustard as a pernicious weed. Bloomfield, No. 4188, June 15, 1916.

Arabis lyrata Lin. var. intermedia (DC), N. Comb.

Arabis ambigua DC. var. *intermedia* DC. Syst. II, 231, 1821.

Arabis lyrata Lin. var. *occidentalis* Wats. in A. Gray Syn. Fl. N. Amer. I, 159, 1895.

Stigma together with very short and thick style about one-third of a millimeter or less; lower one-third of stem with its leaves more or less hirsute. Has been recorded from three places, Bower's Harbor, Grand Traverse Bay and Alpena in Beal's Michigan Flora. I have collected it from Rochester, No. 1533½, July 4, 1896, and from Macomb Co. in vicinity of Disco, No. 4158, May 25, 1916.

HAMAMELIDACEÆ.

A few years ago Dr. Sargent described a new species of Hamamelis, *H. vernalis*, flowering, as the specific name indicates, in the spring. A search for this species in Michigan has not disclosed its presence here. But unlooked for conditions concerning *H. Virginiana* were discovered which may be of sufficient interest to others to have them placed upon record. One of the characters of *H. vernalis* is the red inner surface of the calyx lobes. In *H. Virginiana* the inner surfaces of the calyx lobes are yellow at the beginning of the flowering season, usually in September in this locality, but gradually grow darker until, at the end of the flowering period, which is about the close of the year, most of them are red; all of them are red when vegetation begins in the spring; at least no calyx lobe has been discovered that was yellow on the inner surface at this time. Individual shrubs were found that had red calyx lobes from the beginning and some even had petals with the lower portion red. The particolored petals, however, were not numerous, nor, in all cases, were all the petals of the one flower bicolored.

POMACEÆ.

PYRUS MELANOCARPA (Mx.) Willd.

Said to be frequent throughout the State. Bloomfield, No. 1201, June 15, 1916; Vic. Palmer Park, No. 4403, August 27, 1916.

PYRUS MELANOCARPA (Mx.) Willd. var. ATROPURPUREA (Britton), N. Comb.

Aronia atropurpurea Britton, Man. 517, 1901.

Pyrus arbutifolia var. *atropurpurea* Robinson, Rhodora X. 33, 1908.

Pyrus arbutifolia is credited to Michigan in Beal's Michigan Flora. One of the localities given is St. Clair Co., C. K. Dodge. Dodge's specimens in the Herbarium of Parke, Davis & Co., although distributed as *P. arbutifolia* are of this variety. It is probable that all so-called *P. arbutifolia* collected in Michigan is of this variety and the species should be excluded from the Michigan Flora. In *P. arbutifolia* the calyx lobes are very glandular and the small, ripe fruit is bright red; in *P. atropurpurea* the calyx lobes are glandless or nearly so and the fruit is much larger and purple or black. I have not seen *P. arbutifolia* from Michigan, which is outside of the generally accepted range of the species. The two varieties or species have come under my observation here where they often grow together and it seems to me that *P. atropurpurea* is but a pubescent form of P. *melanocarpa;* the size and form of the leaves and the color of the fruit are essentially the same in both; the berries of the variety are larger than those of the type, globose or occasionally oval. In the species the leaves and inflorescences are glabrous; in the variety more or less tomentose; but there are intermediate forms, and individual shrubs have some of the leaves and inflorescences tomentose while others are glabrous. It seems best, on the whole, to consider *P. atropurpurea* as not specifically distinct from *P. melanocarpa*. Bloomfield, No. 4200, June 15, 1916, and No. 4396, August 22, 1916; Vic. Palmer Park, No. 4276, July 2, 1916, and No. 4406, August 27, 1916 (some parts pubescent and others glabrous).

GERANIACEÆ.

Geranium pusillum, Burm. f.

Banks of Detroit River in Detroit. Considered to be adventive but apparently spreading. No. 4317, July 13, 1916.

TITHYMALACEÆ.

Euphorbia glyptosperma Engelm.

Along the railroad tracks at Farmington, where it is quite frequent. No. 4350, August 12, 1916.

ZIZYPHACEÆ.

Rhamnus cathartic Lin.

A few shrubs are found scattered along a rail fence dividing pasture meadows. Probably from seed dropped by birds. No. 4392, August 22, 1916.

SALICARIACEÆ.

Lythrum Salicaria Lin.

In vicinity of Detroit. No. 4404, August 24, 1916. Probably an escape from cultivation.

CORNICULATACEÆ.

Œnothera muricata Lin. var. parviflora Gates.

A narrow leaved, small flowered form of *Œ. muricata*. Frequent along banks and in fields at Rochester. No. 4415½, Sept. 3, 1916; Belle Isle, Detroit, 195a, August 4, 1912; in a marl bed on Parkedale Farm, No. 3829, August 9, 1914.

Œnothera fruticosa Lin. var. phyllopus Hooker.

This is a form in which there is no distinction between stem and peduncle; the floral leaves are like those of the stem, giving to the flowers the appearance of being solitary and axillary. Freely branched. Detroit, No. 4261, June 30, 1916. Occasional and perhaps an escape from cultivation.

CALCARATACEÆ.

VIOLA PAPILIONACEA Pursh. var. ALBA (T. & G.), N. comb.

Viola cucullata Ait. var. *alba* T. & G. Fl. N. Amer. I. 137, 1838.

Smooth; flowers white. Frequent in places. Near Rochester. No. 3628, May 10, 1914, and near Rockwood, No. 4144, May 21, 1916.

VIOLA ROSTRATA, Ait. var. PHELPSIÆ (Fernald), N. comb.

Viola rostrata Ait. forma *Phelpsiæ* Fernald, Rhodora XVII, 180, 1915.

Flowers white. Near Rockwood. No. 4146, May 21, 1916.

VIOLA PEDATIFIDA, Don.

I have seen living plants of a violet collected by Mr. E. W. Mason of Lapeer, which had palmately many divided leaves, the divisions of which were very long and narrowly linear or filiform. It probably belongs here, where it may be placed until further study will determine its proper disposition. This species has not before been reported from Michigan.

MONOTROPACEÆ.

PYROLA ASARIFOLIA Mx. var. INCARNATA (Fisch.) Fernald.

Leaves orbicular to ovate, rounded, truncate or subcordate at base, dull, smaller than in the specific type which are of a different form also. Common in swamps at Marl Lake, No. 4184, June 11, 1916, and Nos. 4218 and 4219, June 18, 1916, from near Pontiac.

PYROLA ASARIFOLIA Mx. var. ULIGINOSA (Torr. et Gray), N. Comb.

Pyrola uliginosa Torr. et Gray; Torr. Fl. N. Y. I, 453, pl. 69, 1843.

Leaves sub-orbicular to obovate, narrowed to an acute base; smaller than in the preceding variety; not common. Swamps at Marl Lake, No. 4185, July 9, 1916.

POLEMONIACEÆ.

GILIA RUBRA (Lin.) Heller.

Near Farmington, No. 4357, August 12, 1916. Probably an escape from cultivation, but seems to be well established. First discovered, I believe, by Mr. Gladewitz.

LABIATACEÆ.

Ocimum Basilicum Lin. var. glabratum Benth.

Collected near Cass Lake by Mr. C. Billington. Probably an escape from cultivation.

Lycopus asper Greene.

Banks of Detroit River at Wyandotte. No. 4384, August 20, 1917. Has been reported as *L. lucidus* from Port Huron by C. K. Dodge. Lower part of stems prostrate and rooting at the joints.

RINGENTACEÆ.

Penstemon Digitalis (Sweet) Nutt.

Reported from Adrian in Beal's Michigan Flora. I have collected it on Belle Isle, No. 1916, June 20, 1905, and No. 4229 from Algonac, June 22, 1916.

APARINACEÆ.

Gallium Aparine Lin. var. eschinospermum (Wallr.), N. Comb.

Galium Vaillantii D. C. Fl. France IV, 263, 1805.

Galium agreste Wallr. Var. *echinospermum* Wallr. Sched. Crit. 59, 1822.

Galium Aparine Lin. var. *Vaillantii* (D. C.) Koch. Fl. Germ. 330, 1837.

A smaller plant than the type with smaller leaves and fruit. On Parkedale Farm, May 30, 1914, No. 3652; and No. 3964 from Detroit, June 12, 1915.

Galium triflorum Mx. var. viridiflorum D. C.

Stems smooth. Near Marl Lake, No. 4305, July 9, 1916.

Galium Labradoricum, Wiegand.

Probably has been confused with *G. tinctorium.* It is a smaller plant with narrower and shorter, soon reflexed leaves and smaller fruit. Marl Lake, No. 4187, June 11, 1916; Bloomfield, No. 4197, June 15, 1916, and No. 4267 and 4271 at Tacoma, July 2, 1916.

COMPOSITACEÆ.

HELIOPIS HELIANTHOIDES (Lin.) B. S. P. var. MINOR (Hook), N. Comb.

Heliopsis lævis Pers. var. *minor* Hook, Comp. Bot. Mag. I, 98, 1835.

Heliopsis lævis Pers. var. *gracilis* T. and G. Fl. N. Amer. II, 303, 1842.

Stem simple, strict, ending in a single head; leaves very thin, very slightly scabrous. Near Rochester, No. 4416, Sept. 3, 1916.

RUDBECKIA MONTICOLA Small.

A form with the lower leaves of an ovate outline and the upper leaves with a very broad clasping base. Near Rochester, No. 4331, July 15, 1916.

HELIANTHUS SCABERRIMUS Ell.

Found at River Rouge by Billington, Chandler & Gladewitz, who reported it as *H. subrhomboideus* Rydb. The involucral scales, however, are mainly obtuse, which would place it here. No. 4380, August 17, 1916. I have also collected it in Houghton Co., No. 3087, August 24, 1912.

ARTEMISIA LONGIFOLIA Nutt.

Found in considerable quantities near River Rouge. Farwell and Chandler, No. 4379, August 17, 1916.

HIERACIUM AURANTIACUM Lin.

Balast grounds at Detroit, rare, No. 4315, July 11, 1916. Has been found also by Billington, Chandler and Gladewitz.

HIERACIUM VENOSUM Lin. var. SUBCAULESCENS, T. and G.

Near Tacoma, No. 4264, July 2, 1916.

ADDENDA.

The plant mentioned above under *Viola pedatifida* proves to be the *V. pedata* var. *lineariloba,* DC. with very narrow or almost filiform lobes to the leaves.

PYROLA ASARIFOLIA, Mx. var. OVATA, N. name.

Since the *Pyrola incarnata* Fisch. is an Asiatic plant and a variety of the European *P. rotundifolia* Lin., the plant listed above as *P. Asarifolia* Mx. var. *incarnata* should receive a new name and I propose for it the varietal name OVATA.

Parke, Davis Co., Detroit, Mich.

106618

Studies from the Research Laboratory
Parke, Davis & Co.
Reprint No. 144, 1917.

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HOG CHOLERA TRANSMISSION EXPERIMENTS.

By H. Preston Hoskins, Detroit, Mich.
(Animal Pathologist, Research Laboratories, Parke, Davis & Co.)

It would appear to me that some apology is due the members of this association for coming before you on the subject of hog cholera, because, if there is any one subject that has been thoroughly discussed at meetings of this kind during the past five years, it is hog cholera, in all its phases. It so happened that at the time that your very efficient secretary was trying to prevail upon me to make good a promise made him several times, to present a paper, we were having a rather interesting experience with hog cholera in our experimental stables, and this paper is presented for the purpose of reporting this experience and some facts bearing upon the subject of the transmission of the hog cholera virus, artificially and under natural conditions.

There is considerable experimental evidence and practical experience to show that the hog cholera virus is very susceptible to certain environmental influences. From research work reported and from observations made we know that hog cholera virus rapidly loses its disease-producing power under certain conditions. Putrefaction is one of the influences which is important, from a number of standpoints. Hog cholera virus appears to be readily destroyed by the products of putrefaction. Now if this fact be true, without exception, a given amount of hog cholera virus would remain virulent a much shorter time in a hog wallow than it would on the concrete floor of an up-to-date hog barn, other conditions being equal. The virus is resistant to freezing, and to a less extent to drying. Sunlight destroys the virus and different disinfectants vary in their germicidal action against the virus. It might be stated that influences which retard putrefaction prolong the life of the virus, and influences which promote putrefaction hasten the death of the virus.

The English Commission appointed to study certain phases of the hog cholera question carried on a number of experiments to determine the infectiousness of the excretions of cholera hogs and to throw additional light on the subject of carriers of the infection and probable modes of its transmission. If we were to place entire credence in the results of the experiments carried on by the English Commission, it would appear that the dangers of spreading the infection have been greatly exaggerated. As a matter of fact we can not help but be impressed with the great discrepancies which appear to exist between experimental data and actual occurrences.

The report[1] of the English Commission states that, "By placing susceptible pigs on the manure of affected pigs (i. e., litter and fæces soiled with urine) positive results were obtained after the manure had been exposed to natural influences for: No days (i. e., the pigs were placed on the manure immediately the affected pigs had been removed), and for seven days. Negative results were obtained after the manure had been exposed for 1 day, 2 days, 14 days, 21 days, 28 days, and 42 days. By actually feeding fæces only, from infected pigs, after the fæces had been exposed to natural influences, positive results were obtained in no case, but there was a very doubtful result after 21 days' exposure. This doubtful result, however, may be neglected, having regard to the negative results in this connection, and in the experiments with manure (fæces and urine) at intervals of exposure, negative results were obtained after 2 days, 11 days, 21 days, 28 days, 46 days and 54 days."

At the recent meeting of the United States Live Stock Sanitary Association, Dr. Dorset[2] reported the results of some experiments conducted by the Bureau of Animal Industry to determine the infectiousness of hog cholera virus, as carried by persons on their shoes. Briefly, the experiment consisted in placing a number of susceptible pigs in one pen, and a number of cholera infected pigs in another pen, about 100 yards distant. Attendants entered the pen containing the sick pigs, took their temperatures, then disinfected the thermometers and their hands, but not their shoes, and entered the pen containing the healthy susceptible pigs. Contrary to expectations, the latter pigs failed to contract infection during the experiment, which lasted thirty days. The pigs were then moved closer together, 50 yards apart, and the experiment repeated, with the same results, namely: failure to infect the pigs. The susceptibility of the pigs was later proven by inoculation tests.

The outcome of this experiment was somewhat similar to one conducted by the writer while at the University of Minnesota, but not previously reported. A litter of ten pigs was placed in a pen about 50 yards distant from the crematory where we were in the habit of burning, each day, the carcasses of from five to fifteen virus pigs that had been bled and autopsied in serum production work. The original intention was to keep these pigs isolated, and free from infection, if possible, for use in some experimental work later. However, it occurred to us that it would be an excellent opportunity to determine the actual danger of the crematory as a source of infection, criticism having been directed against it on that score. To make a long story short, the pigs failed to contract any infection over a period of several weeks. In fact, we even thought that they were immune, and for that reason removed them to the virus building and inoculated them with a strain of virus of known virulence. They all succumbed to the infection.

It would appear from the above experiments that it should be an easy matter to keep pigs free from infection, even though active cholera cases were near, without a great deal of extra effort. This point is a very important one in connection with experimental work with the disease, where it is almost absolutely necessary to have on hand at all times susceptible pigs for inoculation purposes. Such pigs must be kept healthy and free from infection so that they may be ready for use just as soon as an occasion arises. Further, in inoculation experiments, the necessity for keeping such pigs free from extraneous infection does not terminate with their inoculation, but the pigs must be kept free of such infection during the course of the experiment, or the latter will be valueless.

The following occurrence will serve to indicate the fallacy of the belief that it is easy to control accidental infection, even when conditions approach the ideal. In our experimental stables our susceptible pigs are kept in pens about fifteen feet square, the pens being completely separated from each other by solid concrete walls. The pens have concrete floors and are entered from a common passage-way through a door with a sill a foot high, and a foot in width, separating each pen from the passage-way. Each pen has a double door, the outside door being solid and of wooden construction, and the inner one being a screened frame door.

Each pen has a window opening on the outside of the building, and is screened.

The attendant who looks after the pigs is furnished with rubber boots and rubber gloves, and before entering a pen, containing either sick or well hogs, thoroughly disinfects his boots and gloves by immersion in a strong disinfectant solution, a 2% solution of which has been determined to kill the hog cholera virus by contact in five minutes.[3] The same process is carried out on leaving a pen.

Several months ago we had on hand two lots of normal susceptible pigs, six in one group and ten in the other, awaiting an experiment. On the afternoon of Dec. 14th, 1916, the attendant reported that the pigs in the group of six were not acting well. On taking their temperatures, they were found to range from 103.5°F to 106.4°F. A sample of blood was taken from each pig, and then each pig was given a fairly large dose of hyperimmune serum. About a week later, on Dec. 23rd, to be exact, the attendant reported that the second group of pigs, ten in number, were showing slight symptoms. Temperatures were taken and they were found to range from 103.4°F to 106.6°F.

Although the indications were highly in favor of the trouble being hog cholera, it was decided to determine positively that this outbreak was due to the filterable virus, and for this purpose the defibrinated blood from pigs 340 and 343 was diluted with several volumes of sterile, normal saline solution, passed through a Berkefeld filter, and then inoculated into susceptible pigs, with the following results:

> Pig 376: Inoculated with filtered serum from pig 340, showed a febrile reaction on the 5th day, the temperature rising to 106.7°F on the 7th day, with clinical symptoms being shown. Lived until 19th day, although very sick for five days. P. M.: Lesions in lymph-nodes, kidneys, intestines, lungs, bladder and larynx.
>
> Pig 377: Inoculated with filtered serum from pig 343, showed a febrile reaction on the 5th day, when the temperature reached 106.5°F. Clinically ill on the 7th day, and killed on the 11th day to secure the virus. P. M.: Lesions in kidneys, spleen, larynx, intestines and lymph-nodes.
>
> Pig 340: Reported as sick on Dec. 14, 1916. The following day a blood sample was drawn and the pig given 55.0

c.c. of anti-hog cholera serum. The pig developed a case of chronic cholera and died Jan. 22, 1917. Cholera lesions at autopsy.

Pig 343: Reported as sick on Dec. 11, 1916. Blood sample drawn the following day and 60.0 c.c. of serum administered. Temperature dropped and reached normal Dec. 25, 1916. Recovered.

Bacteriological examination of the blood from a number of the pigs showed the blood to be free from those organisms usually associated with the filterable virus disease. These tests and examinations proved pretty conclusively that we were dealing with true hog cholera, caused by the filterable virus.

There is not much doubt, however, that the infection came from some one of the subacute experimental cases in the stable early in the month of December. Based upon some observations previously made by the author,[4] it appeared that the pigs in group 1 were exposed to infection about Dec. 10th, and those in group 2 about Dec. 20th. (The strain of virus in use in the laboratory just prior to this outbreak of hog cholera was the same strain used in the production of serum at the Minnesota State Serum Plant, kindly furnished by Dr. H. C. H. Kernkamp.)

The following tables give a summary of the data concerning the two groups of pigs:

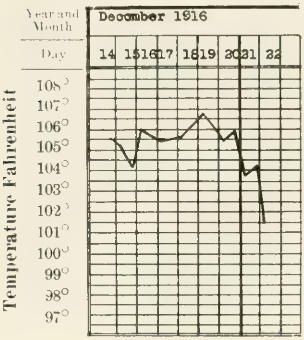
GROUP I.

Tag Nos.	Date Rec'd	Temp. 12-14-16	Highest Temp.	Date	Outcome
332	9-2-16	105.6	106.7	12-16-16	Died 12-23-16
338	"	105.6	107.0	12-18-16	" 12-23-16
340	10-13-16	106.4	106.5	12-17-16	" 1 -22-17
341	"	106.0	106.9	12-16-16	" 12-30-16
342	"	105.2	106.0	12-15-16	" 12-24-16
343	"	103.5	106.4	12-17-16	Recovered.

GROUP II.

Tag Nos.	Date Rec'd	Temp. 12-23-16	Highest Temp.	Date	Outcome
348	11-7-16	106.5	106.5	12-26-16	Killed 12-29-16
349	"	104.8	107.0	12-26-16	Died 1- 5-17
350	"	106.6	107.0	12-26-16	" 1-18-17
351	"	106.4	107.0	12-29-16	" 1-16-17
352	"	103.4	106.7	12-28-16	" 1-16-17
353	"	106.2	108.2	12-26-16	" 1- 8-17
354	"	106.6	107.2	12-26-16	" 1- 1-17
355	"	105.0	107.5	12-29-16	" 1-22-17
356	"	105.6	107.0	12-28-16	" 1-14-17
357	"	105.3	108.3	12-25-16	" 12-31-16

The following charts show the temperature curves of one representative pig from each group (Nos. 338 and 398) and the two pigs used to prove the infection due to the filterable virus (Nos. 376 and 377), as well as the clinical observations made and the post-mortem findings:

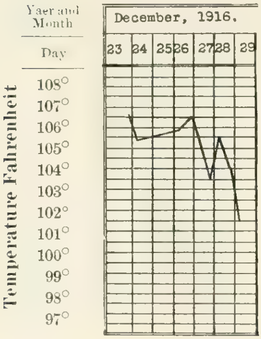


Hog No. 338.

REMARKS:

Dec. 14. Appears sick.
15. Bled from tail.
23. Found dead.

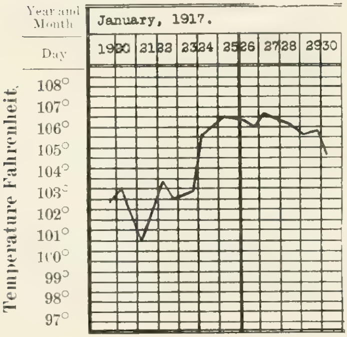
Autopsy: Subcutaneous tissue and musculature studded with hemorrhagic extravasations. Heart extensively petechiated. Pneumonic areas in lungs, also petechiae. Icterus noticeable throughout cadaver, due to occulusion of bile duct with ascarides. Kidneys petechiated. Bladder petechiated. Larynx icteric and hemorrhagic. Petechial hemorrhages in serosa of intestine. Lymph nodes throughout body very congested.



Hog. No. 348.

REMARKS:

Dec. 23. Sick.
29. Moribund. Killed and autopsied. Lungs show lobular pneumonia. Mediastinal lymph nodes enlarged and slightly hemorrhagic. Lobules of liver very distinct; ascarides in bile ducts. Many ascarides in intestinal tract. Kidneys congested. Bladder shows intense injection of capillaries in mucosa. Spleen enlarged and soft. Ileo-caecal valve reddened.



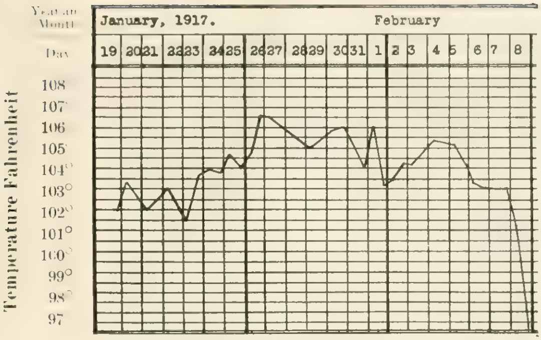
Hog No. 377.

REMARKS:

Jan. 19. Intramuscular injection 20.0 c. c. filtered serum of Hog No. 343.
26. Anorexia.
30. Very weak. Killed by bleeding from carotid artery.

Autopsy: Lungs normal. Kidneys hemorrhagic. Bladder hyperemic. Spleen slightly enlarged. Stomach hemorrhagic. Ileo-caecal valve inflamed. Lymph-nodes enlarged and slightly congested. Slight hemorrhages in larynx.

Hog. No. 376.



REMARKS:

Jan. 19. Intramuscular injection 10.0 c. c. filtered serum of Hog No. 340.
26. Anorexia.
Feb. 3. Very sick.
6. Moribund.
8. Found dead.

Autopsy: Lungs show lobular pneumonia. Mediastinal lymph nodes enlarged, and very hemorrhagic. Liver appears normal. Spleen enlarged and soft. Kidneys petechiated. Bladder badly congested. Intestines hemorrhagic. Larynx hyperemic.

The viability of the hog cholera virus is apparently well illustrated by the following instances which came to the personal attention of the writer several years ago, while located in Minnesota. Hog cholera mysteriously made its appearance in a small herd of hogs on a large farm in the southeastern part of the State, early in the spring. This farm was somewhat isolated, being fourteen miles distant from the nearest railroad station, although a spur used only for freight purposes extended to a point only a few miles away. There had never been any hog cholera on the premises so far as known, and there had been none in the county since the preceding fall. No new hogs had been introduced on the farm for quite a while.

After investigation of the outbreak, in the hope of being able to trace the infection, about the only plausible clue was the following: About two weeks prior to the appearance of cholera on the farm, a carload of drain tile had been unloaded on the railroad spur previously mentioned and hauled to the farm in wagons. The manager of the farm had assisted in unloading the tile, and he was in the habit of looking after the hogs, which were pure-bred Poland-Chinas. He informed me, on my visit to the farm, that the tile had been shipped in a stock car and that there was considerable litter in the bottom of the car. The assumption was that this car, previous to being loaded with the tile, had been used to haul hogs and that these hogs had contaminated the litter in the car. Either some of this litter had adhered to the tile and thus been conveyed to the farm, or some of the men had carried the infection on their clothes or shoes. All of which only further emphasizes the importance of the proper disinfection of stock cars.

One other instance of the apparent ease with which the disease may be transmitted will be cited. Hog cholera made its appearance on a farm in the west central part of Minnesota a few years ago in the middle of winter. Upon investigation it was found that there was no cholera in the neighborhood, there had been none on the farm so far as known, and none of the usual modes of infection seemed to be even likely in this case. However, upon questioning the owner of the hogs, the information was gained that a relative from an adjoining state had visited him at Christmas time, and the relative had had cholera on his farm at home quite recently. About the only solution that seemed reasonable was that this relative had carried some infection, very likely on his shoes, from one farm to the other, several hundred miles apart.

It must be remembered in any such cases that the disease must be identified and the diagnosis proven, to be of any scientific value. However, in the two cases mentioned, both of which came to the personal attention of the writer, there was no reasonable doubt as to the true nature of the disease. On the other hand, it is absolutely impossible to prove definitely that in a given outbreak the infection was carried in this, that or the other way. Very often it is a process of elimination, and we must content ourselves with selecting what appears to be the most likely avenue of infection.

It very frequently happens, in trying to explain to our clients how infection is carried, that our theories appear very far-fetched, and when we take into consideration the sometimes mysterious manner in which a disease such as hog cholera makes its appearance, it is no wonder that we still have among the laity those who believe in the spontaneous origin of these diseases, or that they are due to dietetic errors, meteorological disturbances or some act of Providence. We are often helpless when it comes to enlightening them, or powerless to make them see the error of their ways.

In any experiments on the transmission of hog cholera there are certain fundamental requirements which should be complied with, in order that no flaws can be detected in the experiment. Some of these conditions are much easier to meet than others. In inoculation experiments the hardest factor to control is the accidental infection of the hogs in the experiment. It is usually customary to place with the inoculated pigs a number of control animals that are not inoculated. These controls should not contract hog cholera immediately, but in the event of positive results in the inoculated animals, these should transmit the infection to the controls by contact or association. Negative results are frequently confusing. An animal which does not become infected as the result of the inoculation with suspected material, should subsequently be tested for immunity by inoculations with material of known virulence. Failure to respond to the second inoculation may be due to immunity which existed at the time of the first inoculation, or actually as the result of the first inoculation which had the effect of vaccinating the animal.

In a previous publication[4] I have called attention to the great variations in the amount of resistance to infection shown by different hogs, varying from one extreme to the other, namely, complete susceptibility to what appeared to be complete immunity. In the latter cases the possibility should be kept in mind that these apparently immune animals may not have been immune in the true sense of the word, but these hogs may actually have passed through a reaction, so slight, however, as not to be noticed by the rather superficial means of detection at our command.

Fresh in our memories is the 1914 outbreak of foot and mouth disease in this country, the origin of which has never been explained or the portal of entry definitely ascertained. There is not one of us, however, who doubts for a minute that the virus of the dreaded plague was transported in some way to the point where the outbreak had its start. During the past summer, when infantile paralysis was rampant in certain sections of the country, isolated cases cropped up here and there, without warning, and frequently with no apparent connection, direct or indirect, with a previous case of the disease. Physicians in many instances could do nothing but make the wildest guess as to the probable manner of convection of the virus.

Since the epochal discovery of Dr. Theobald Smith and his associates, of the role of the cattle tick in the transmission of southern cattle fever, no material progress has been made in the study of disease transmission. There must still be some secret that awaits discovery by which we may be able to satisfactorily explain some of the rather baffling phenomena which all too frequently present themselves to those of us who are engaged in the study, treatment and control of infectious diseases.

There is a phase of the question which is important to us as veterinarians, especially those engaged in country practice or hog cholera control work, federal, state or county. For the past five years a great deal of educational work has been carried on, not only in the great hog-raising states, but in practically all of our states, taking information to the farmer on the subject of infectious diseases. Especial emphasis has been placed upon the ease with which the infection of hog cholera may be spread from farm to farm. Undoubtedly a great amount of good has been accomplished in this way, and there is not one of us who doubts that less infection has actually been spread as a result of this educational propaganda.

On the other hand, the fact must not be lost sight of that much of the good accomplished by this work may be undone by too much publicity given to negative results obtained in a small number of experiments. No matter how carefully such work is planned, and no matter how conscientiously the experiments are carried out, there still remains the possibility for errors to creep in, or the absence of some factor from the artificial conditions in the experiment, not usually absent under natural conditions on the average farm. The agricultural press is usually hungry for and eager to publish anything relative to investigational work with animal diseases, and the wrong interpretation is sometimes placed upon the publication of results of work conducted in our scientific laboratories. For this reason great care should be exercised in drawing conclusions and publishing the results of research or experimental work of the kind referred to.

SUMMARY.

To briefly recapitulate, the facts presented in this paper are substantially as follows:

1. That on two occasions, outbreaks of hog cholera in Minnesota had apparently been the result of the conveyance of the infection by intermediate objects for distances of several hundred miles.

2. That in the experiment conducted at University Farm, it had been shown that susceptible pigs could be kept close to extensive infection without necessarily contracting the disease.

3. That the results of this experiment were practically duplicated by the experiments of the Bureau of Animal Industry, reported by Dr. M. Dorset at the recent meeting of the United States Live Stock Sanitary Association, in Chicago.

4. That in spite of the utmost precautions to prevent the accidental infection of experimental pigs, kept in our Detroit laboratories, on two occasions recently, different groups of pigs became infected with hog cholera.

5. That this accidental infection was proven by appropriate tests to be due to the filterable virus, and the affected pigs in most cases proved to be unusually free from secondary invading organisms commonly met with in this disease.

6. That serious discrepancies exist between the results of different experiments, as well as between results obtained under experimental and natural conditions.

7. That much good already accomplished may be undone by the premature publication of negative results of transmission experiments, when all our teachings during recent years, especially to the laity, have been to the effect that hog cholera is a disease that is very easily transmitted.

REFERENCES.

1. Final Report of the Department Committee, etc., Part IV, London, 1915.
2. Dorset: Proc. United States Live Stock Sanitary Association (1916).
3. King and Drake: Jour. Amer. Vet. Med. Asso., 48, 3 (1915), pp. 315-316
4. Hoskins: Jour. Amer. Vet. Med. Asso., 49, 6 (1916), pp. 817-829

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